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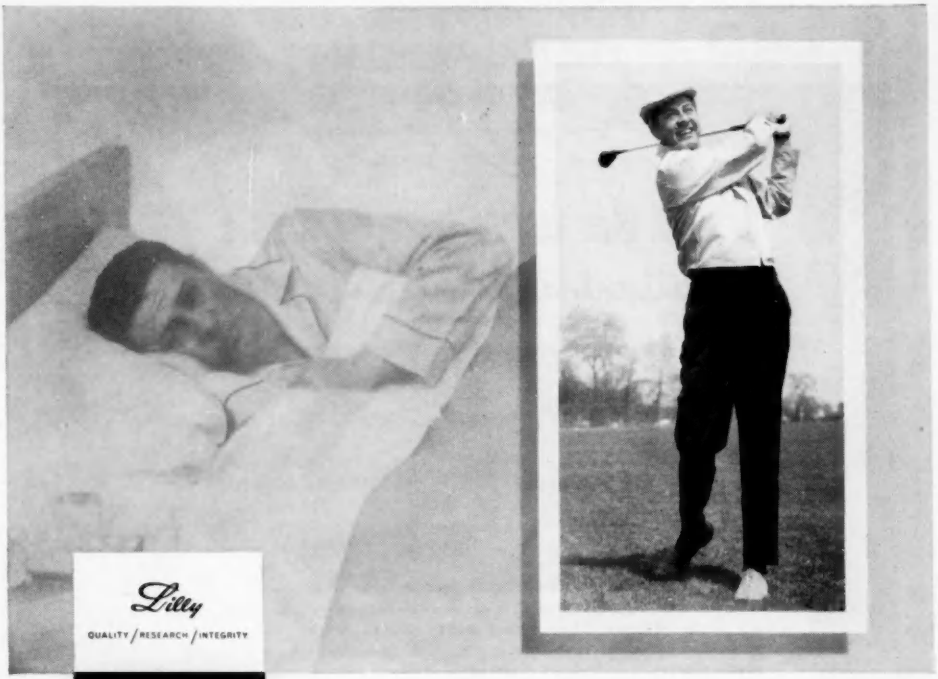

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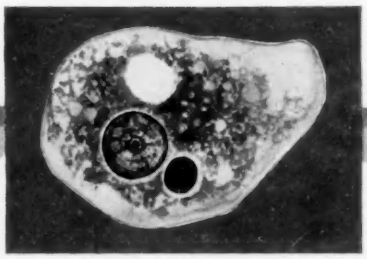

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Vol. 6 *July 1957* *No. 4*

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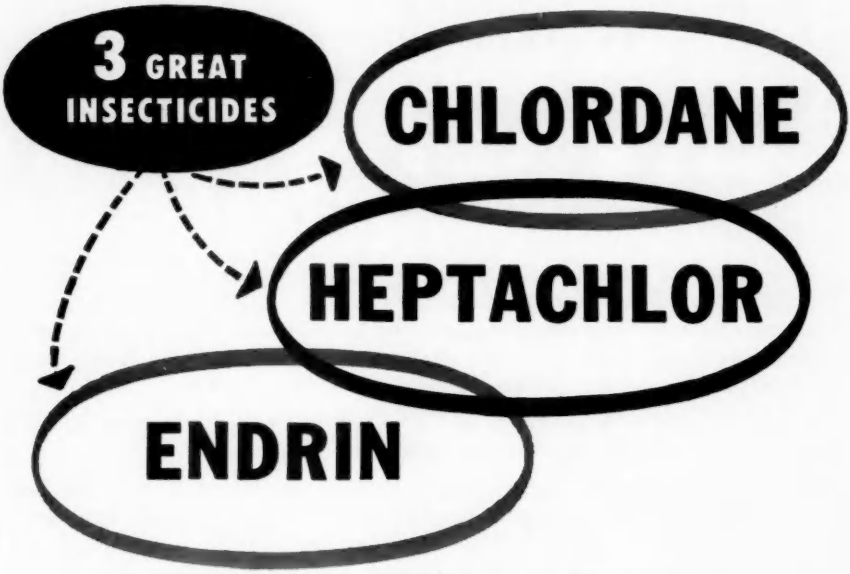

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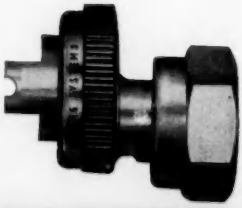

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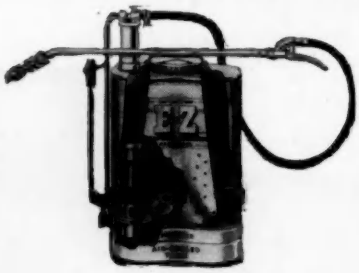

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JOHN M. HENDERSON

Communicable Disease Center, Bureau of State Services, Public Health Service, Savannah, Georgia

As indicated by the title, this article deals with the control of vectors. None of its content applies to the eradication of any vector species, which involves quite different criteria.

According to one dictionary, a vector is an "organism, usually an insect, which carries and transmits disease-causing organisms." This definition is what has come to be known as the classic definition of a vector.

Some 20 years ago the number of arthropod species which had been incriminated in the transmission of various infections of man in this country, and hence were classic vectors, was rather lengthy. But from the standpoint of control, interest was chiefly centered on only four species, all insects. These were the house fly and three mosquito species: *Aedes aegypti*, *Anopheles quadrimaculatus* and *Anopheles freeborni*.

The vectorial status of these four species was clear-cut. *A. quadrimaculatus* not only could, but did, transmit malaria at the rate of hundreds of thousands of cases annually. *A. freeborni* also could and did transmit malaria to a considerable extent. An epidemic of *aegypti*-transmitted dengue fever swept through at least six southern states in 1934, with an estimated 25,000 cases in Miami and Savannah alone (Griffitts, T. H. D. *et al.*, 1935; McDonell, G. M., 1935). Few if any would question the continuing role of the house fly then and now in the transmission of endemic enteric infections.

On the other hand, it might be postulated that twenty years ago one vector already had passed from the "could and did" stage to the "could but did not" stage. This was *Culex quinquefasciatus* in the Charleston, South Carolina area, were filariasis formerly was endemic. Accordingly, the fate of this species may serve as a forerunner of things to come.

What is the picture today as compared with yesterday? In the first place, there has been considerable rearrangement in the list of primary classic vectors. Practically speaking, the two anopheline species have reverted from a position of "could and did" to one of "can but do not", transmit malaria. No epidemics of dengue since 1934 are recalled and there has not been even a localized outbreak of yellow fever since 1905. Although the hazard of recurrence of both of these viral infections may be very real, with our present knowledge and armamentarium probably no one would concede the possibility of more than small scale outbreaks of *aegypti*-transmitted yellow fever. With improved sanitation and decline in enteric disease incidence, no doubt fewer cases of these infections are transmitted by the house fly today. Yet in relative importance this insect probably has moved to the top of the list, mainly due to the change in status of *A. quadrimaculatus*.

In the second place, developing knowledge of the arthropod-borne viral encephalitides in this country has greatly expanded the list of mosquito vectors

of disease. To the list of primary vectors which can and do transmit disease in quantity should be added *Culex tarsalis* and probably *Culex quinquefasciatus* the former having greater significance in California and the latter in Texas. Twenty-one arthropod species have been found naturally infected with one or more strains of encephalitis virus, of which 15 are mosquitoes. These include species which apparently can and occasionally do transmit encephalitis to man and others which can but at least normally do not for one reason or another.

In the third place, the scope of public health has been greatly broadened in this country during the 20-year period, at least in the minds of some people and in some areas. Concepts of the scope of arthropod control in the interest of public health have not been exempt from this trend; especially that of mosquito control. As Andrews puts it in referring to Alaskan mosquitoes: "But whether these creatures spread disease from lower animals to man, or from man to man, or whether they have nothing whatever to do with spreading disease, their overpowering numbers and vicious biting habits make insect control an almost necessary prelude to land development. To imply that they are unimportant from a health standpoint simply because they are not known to transmit infectious organisms is to discredit the basic tenet of the World Health Organization Charter. Thus, insect control becomes an essential function of health organizations in bringing about relief from severe insect pestiferousness."[1]

In consequence, the term "arthropods of public health importance" is finding its way into the terminology of public health practice. As interpreted by the Florida Legislature and State Board of Health, for example, this includes any arthropod problem which importantly interferes with human comfort and well-being, i.e., the socioeconomic status of the people of Florida. In effect this represents an evolution in the concept of the meaning of "vector," which in time may find broad recognition in language usage.

As a result of this process of relating classic vectors, quasi-classic vectors, potential classic vectors and sheer comfort-destroying development-impeding organisms to a common socioeconomic reference plane, state and local health agencies in Florida, for example, are giving great attention to the control of the salt marsh mosquito *Aedes taeniorhynchus*, and negligible attention to *A. quadrimaculatus* (Mulrennan, J., 1956, personal communication). Yet this salt marsh mosquito has not been found naturally infected in this country with any microorganism infectious to man. On the other hand, the importance of controlling the house fly, a classic vector, is receiving full recognition consistent with the State Board of Health's policy of emphasizing permanent control measures.

Only the more theoretic aspects of vector control have been discussed so far. In this country, the financing and management of modern vector control programs are primarily local responsibilities. What then is the practical effect of broadened concepts of vector control on the establishment, maintenance and operation of local programs? A prime example of the classic public health advantages of an integrated comprehensive mosquito control program is provided

[1] Excerpt from Foreword by Justin M. Andrews, Associate Chief for Program, BSS, USPHS, to The Biology of Northern Mosquitoes. Frohne, W. C. *Public Health Reports* **71**(6): 617 (June 1956).

by California, with its 55 local mosquito-abatement agencies, its thirty thousand square miles of active control area and its three and a half million dollars of local funds appropriated annually for mosquito control.

California is unfortunate in that it has a higher average annual incidence of reported mosquito-borne infections than any other state. Its major mosquito problem species are *Culex tarsalis* and *Aedes nigromaculis*. *C. tarsalis* has been epidemiologically established as the major vector of human encephalitis in California and is also the cause of moderate discomfort. *A. nigromaculis* is the cause of extreme discomfort due to its aggressive behavior and high seasonal prevalence; as a discomfort problem it approaches that of certain Alaskan mosquitoes. While found naturally infected with encephalitis virus in Colorado, *A. nigromaculis* is credited with limited to negligible importance in encephalitis transmission in California. The irrigation of eight million acres of land in California is the primary factor responsible for the prevalence of both species, although others are significant. Both species are commonly found in the same irrigated field system, although their proportions and numbers may vary widely in different individual pools. Drainage and improved irrigation practices, which are stressed, commonly achieve reduction of both species, although selectivity is possible. The same may be said of chemical control.

There is general agreement that somewhat more effective control of *C. tarsalis* could be realized if a given amount of money were to be expended for the control of this species alone, rather than both species. On the other hand, there is essentially unanimous agreement in California that local support for organized mosquito control on a continuing basis in the affected areas is predominantly based on the desire for relief from acute discomfort and that the amount of funds appropriated and the number of local mosquito control agencies would be drastically reduced if anti-mosquito operations were to be restricted to *C. tarsalis*.

Under the circumstances, maintenance of the present level of control against the classic vector is made possible only by a comprehensive attack against all mosquitoes of public health importance. Likewise, the development through research of improved methods of control for *A. nigromaculis* (the greater operational problem), offers significant reflected knowledge useful in the control of *C. tarsalis* in their common habitats.

It is not advocated under this philosophy of comprehensive control that all vector species of public health importance should be controlled at all times and places. Rather, selectivity and flexibility are core elements. They should be controlled or prevented at various times and places only when the problem or the realistic threat is important enough to warrant the effort. Conversely, the justification for and management of anti-vector operations in any local area should be related to their total public health importance rather than solely to their current or historic status as classic vectors, and the supportive functions of state and Federal health agencies should be geared to this more comprehensive need.

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ANOPHELES GAMBIAE IN RELATION TO MALARIA AND FILARIASIS IN COASTAL LIBERIA

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The only previous work on the vectors of malaria and of filariasis in Liberia was carried out by Gelfand, who studied the mosquitoes concerned with bancroftian filariasis (1955a) and compared *Anopheles gambiae* and *A. melas* both as to their bionomics and as to their efficiency as vectors of malaria (1955b).

The present study is based on nightly collections made from June 1, 1955, to July 24, 1956, and is concerned only with *A. gambiae*. A deliberate effort was made to exclude *A. melas* from the data. Since adult females of *melas* cannot be distinguished from those of *gambiae*, only those collecting sites were used, with the exception noted under the section on filariasis below, where *melas* was not present, as determined through egg identifications.

COLLECTING STATIONS

The seventeen collecting stations affording data for this study are shown on the map, Figure 1. The area mapped lies on the west coast of Africa from 4 to 16 miles inland. The Institute is approximately at 6° 12′ N, 10° 22′ W. Insect ecology in Liberia has been discussed by Briscoe (1952). Only at December village were collections made throughout the entire project. All the other sites were visited for a variable number of weeks as shown below; during any one week collections were made at from 6 to 12 of these sites.

Site	No. weeks collected	Approximate no. houses
Beercase village	16	4
December village	60	30
Farwein village	49	25
Gblogblo village	21	6
Jallah village	44	10
James village	6	5
Jerri village	8	7
Institute camp	12	2
Logan village	16	8
Musah village	29	6
Peabody village	26	8
Peter Manley village	40	30
Sammy village	57	10
Smell-no-taste village	8	40
Snafu village	12	12
Tar village	41	5

The lower reaches of both rivers are bordered with mangrove swamp and

FIG. 1. Map of the vicinity of the Liberian Institute, Marshall Territory, Liberia, showing collecting stations. Numbers show the rate of filarial infection found in mosquitoes collected at each station.

Snafu village lies in this type of ecologic environment. Inland of the mangrove swamp is a belt of sandy savannah running east and west. This does not follow the rivers inland and only Sammy village lies within it. All other stations lie on somewhat higher ground in cut-over rain forest.

All these places except Snafu, which was not used in the malaria data, are well removed from the normal breeding water of *A. melas*, and only *gambiae* eggs were laid by captured females. *A. melas* breeds in the brackish pools in the mangrove between daily high tide and semi-monthly spring tide levels. During the rainy season, roughly from the middle of May to the middle of October, about one third or more of the total ground surface is swampy or under water. In the dry season the swampy ground is reduced to about one-fourth of the total surface. The numerous small streams never dry up entirely and there are many permanent swamps of various sizes.

COLLECTING TECHNIQUES

The three collecting techniques used for this study were similar to those utilized by Gelfand (1955b). Throughout the first twelve months of the 60-week period six collectors were employed, usually organized into two teams but sometimes into three. Accordingly, depending on the organization, collections were made at two or three different sites each night. The three-man team made possible the use of all three techniques: the man-baited trap, hand-collecting on human bait in the open, and hand-collecting of the mosquitoes resting in native houses each morning. When the team consisted of two men, the trap was not used. During the last eight weeks four collectors were used as two teams.

The trap used consists of a light, strong frame work 3½ feet wide, 7 feet long and 5 feet high, covered with screening outside of which are mats made of woven palm. One end serves as a door and is protected by a double cloth which can be closed and tied shut from the inside. The roof is a canvas tarpaulin. On the long sides, about four feet from the ground, are louvres five inches deep with a seven inch outside opening and a half inch inside slot running the full length of the side. Palm mats are not placed above the louvre. Portability was a major consideration in the design of this trap and no attempt was made to simulate the construction of a native hut, on the conviction that the bait is more important to the mosquito than the bait's environment. The trap was constructed to fit the bed of a pick-up truck, light enough to be handled easily by the collectors and strong enough to withstand the constant handling. It was operated from sunset to sunrise with a collector sleeping within on a folding cot. Before leaving the trap in the morning the collector caught and caged any mosquitoes found inside.

For the night collecting on human bait, two men stripped to the waist were stationed in clement weather on a bench in the open at least 20 feet from the trap and from the nearest hut. During rain they were stationed under the cover of the open porch found on many native houses. Armed with flashlights and test tubes, these collectors caught and caged the mosquitoes attempting to feed on them during the night. Each morning, as soon as the local inhabitants were up and about, the collectors surveyed the huts at the site and caged all mosquitoes found. In villages consisting of fewer than ten huts, all huts were surveyed if possible. In larger villages collections were made in ten huts each time.

Because of the construction of the native huts in this region it is nearly impossible even for the most experienced and conscientious collector to find more than a percentage of the resting mosquitoes by direct hand-catching. The roof comes to a high central peak with the thatch tied to the outside of a pole framework. At ceiling height there is a loose framework of poles, giving the mosquitoes ready access to the under surface of the thatch but making it quite difficult for the collector to find them. The majority of mosquitoes recorded from houses were found on the walls and on objects within the rooms. Since the mosquitoes were wanted alive, knock-down spraying was not used.

The mosquito collectors were kept on a drug therapy regime with weekly doses of pyrimethamine in order to avoid contaminating the data. In a few instances

gambiae from the laboratory colony were given test feeds on collectors as an additional check, but these feeds invariably failed to produce infected mosquitoes.

WEATHER AND THE *A. GAMBIAE* POPULATION

Records from the weather station at Robertsfield[1] for the seven years from May, 1949, to April, 1956, show an annual average rainfall of 160.09 inches. Between May, 1953, and April, 1954, there were 209.20 inches of rain. Between May, 1949, and April, 1950, only 129.94 inches fell. Of the sixty-four months involved in the foregoing record, only four months had less than one inch of rain: January, 1953; January, 1956; February, 1950; and March, 1951. Although the Liberian Institute is located only three miles from Robertsfield, rainfall records kept here during 1956 and compared with the Robertsfield records for the same period indicate that the Institute receives up to ten per cent more rain than does the air field.

As a result of this abundant rain adequate breeding water for *A. gambiae* is to be found in this area throughout the year. This in turn enables the continuous transmission of malaria and filariasis, as the data presented below will show.

In Figure 2 the weekly rainfall records from Robertsfield are plotted graphically for the period of this study, June 1, 1955, to July 24, 1956. For the same period the maximum and minimum temperatures and estimated weekly averages taken from a thermographic record maintained at the Institute is plotted. Note that the estimated weekly average temperature is not the arithmetic average between the maximum and minimum temperatures but was estimated visually from the weekly thermographs by describing a temperature line having as much of the graph above it as below. This minimizes the effect on the average of the occasional brief temperature extreme.

The number of *gambiae* captured weekly per man-night is plotted on Figure 2 for direct comparison with temperature and rainfall records. To arrive at the capture per man per night, the total number of *gambiae* collected during a week at the stations under consideration was divided by the total number of man-nights involved. This seems the best method of computation as during any week the trap, and thus the three-man team, might be used at some stations but not at others, where only the two-man team would be employed. This irregular alternation of collecting methods was usually necessary because the trap must be transported by truck and some of the stations used could not be reached except over trails not practicable to vehicles. Furthermore, collections might be made at certain stations several times and in a few cases as many as four times a week.

These man-night figures show that *gambiae* is present in greatest numbers during the rains but persists during the dry season and never disappears. Peaks in mosquito catches follow the peaks in rainfall by about a week, except when the weekly rainfall includes a severe cloudburst—as, for example, during the week of June 13, 1956, when 9 of the 13 inches fell within one 16-hour span—when

[1] Kindly furnished by Mr. Derek Winter, meteorologist for Pan American World Airways stationed at Robertsfield.

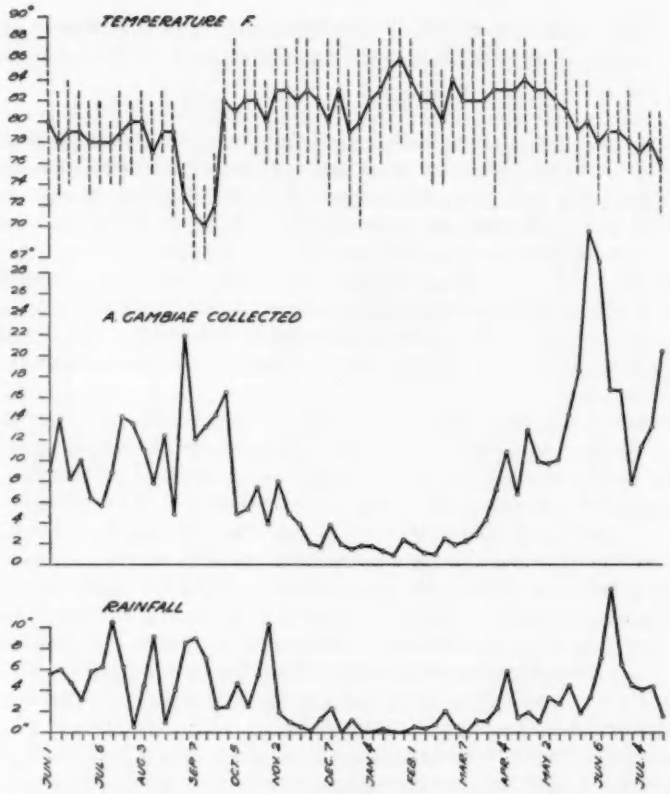

FIG. 2. Rainfall in inches, temperature fahrenheit (maximum, minimum and average), and *A. gambiae* collected per man night for each week during the period studied.

many of the puddles and small ponds where larvae are developing are flushed out, setting back the adult production for the following week.

Comparison of the weekly temperatures with mosquito catches indicates that temperature also seems to influence mosquito production, as peaks in mosquito population coincide with downward trends in the temperature and sharp drops in population frequently correspond with a general rising trend in the temperature. Note, for example, the sharp population drop between the week of September 28 and the week of October 5 as indicated by the decrease in collecting from more than 16 *gambiae* per man per night during the former week to less than 5 per man per night during the latter week. Rainfall continued at more than two inches a week for still another month, so that there is no question that adequate breeding waters continued to be available. However, it is to be noted that during the weeks of September 14 and 21, immediately prior to the population decline, the mean temperature rose from 70° to 82°. Similarly, the high peak

of population in May follows the declining temperatures experienced during that month, although here a relationship is less obvious since both rainfall and population had been rising during the previous two months although the temperatures had remained high. It is possible that the higher temperatures during the dry season are as important as the reduced but still adequate rainfall in limiting the *gambiae* population, since rainfall is never reduced to the point where suitable breeding waters are eliminated or even seriously curtailed. In the laboratory colony of *gambiae* we have observed that during the dry season the mosquitoes live longer but lay fewer eggs and experience a higher larval mortality in a situation where they are completely protected from the influence of rainfall as such. Furthermore, we can reduce mortality and increase egg production in the colony during the dry season by cooling the rooms somewhat. The precise nature of the influence of temperature is not clear but is probably indirect. Figure 2 suggests that the absolute temperature may be less important than the general relative change in temperature.

COMPARISON OF COLLECTING METHODS IN POPULATION SAMPLING

The usual method of collecting malarial mosquitoes is by hand-catching or spray-catching those resting in native houses. This has the advantage of being convenient and rapid where the primary object of the collecting is to assess malaria prevalence through salivary gland dissections. Spray-catching has the additional advantage that more mosquitoes are collected where, as here, a significant portion of the normal resting surfaces are not convenient for hand-catching, but it is of no value where the mosquitoes are wanted alive. Since the native huts afford free ingress to mosquitoes, free egress is afforded as well, so that only that part of the population will be sampled which remains in the house.

The man-baited trap described above allows ready ingress but prevents egress of mosquitoes entering to feed, thus the sample obtained is not limited to those remaining to rest but includes all entering to feed. That the trap does not simulate the design of the native huts seems to be of no importance as shown by the ample catches made with it. The only difficulty encountered in the use of the trap was to ensure that the collector assigned to man it actually remained inside from sunset to sunrise. An effort was made to ensure that he did not swathe himself in a cocoon of blankets, thus impairing his usefulness as bait.

The two-man team collecting in the open was quite satisfactory, for the catch includes all mosquitoes attempting to feed on the collectors, and is probably the best method for sampling the man-biting species, since those hesitating to enter or shunning houses will be included. Some difficulty was experienced ensuring that the collectors remained on the job for the assigned period and did not go to sleep or wander off to join local villagers in various group or individual recreational activities. Frequent spot-checking at random times of the night, with severe penalties for transgressors, was found advisable to keep the program going properly.

The results produced by the three collecting techniques are summarized month by month in Table 1 and these data are presented graphically for ready

TABLE 1

Monthly summary of A. gambiae *collected by three methods*

Month	Total	per man	Man-baited trap		In the open		Hut resting	
			total	per man	total	per team	total	per team
July, 1955	1228	10.0	577	14.0	407	9.9	244	6.0
August	1264	10.0	499	13.5	565	12.6	200	4.4
September	1920	15.1	365	12.6	1057	21.6	498	10.2
October	828	6.3	177	5.2	433	8.8	218	4.4
November	571	4.4	116	3.9	352	7.0	103	2.0
December	205	1.7	45	1.5	125	2.7	35	0.8
Jan., 1956	186	1.6	32	1.3	111	2.4	43	0.9
February	173	1.6	47	1.7	99	2.4	27	0.6
March	448	3.0	25	1.7	305	4.6	118	1.8
April	1186	10.0	323	10.4	609	13.8	254	5.8
May	1893	15.4	269	10.0	1095	22.8	529	11.0
June	1703	19.8			1040	24.2	663	15.4
July	936	13.4			568	16.2	368	10.5
13 months	12,541	8.14	2475	7.56	6766	11.16	3300	5.44

comparison in Figure 3. The figures for the trap are based on the catch per man per night, found by dividing the total mosquitoes trapped during a month by the number of times the trap was used. The night collecting and the hut collecting are, however, treated on a per-team basis, reached by dividing the total monthly catch by the number of nights worked by the two-man night teams, or by the number of times a collecting team surveyed the huts at a site.

From the table and the graph it is clear that, under conditions pertaining here, night collecting in the open produces the greatest numbers of live *A. gambiae* and despite some supervisory inconveniences, the population sample collected by this method is entirely proportional to that obtained by the other methods used. Supervisory difficulties doubtless account for the fact that more *gambiae* were taken in the trap than by night-collecting during July and August 1955. By September the night collectors were well enough trained and supervised so that the relative value of the method could be established. It appears that any of the three collecting methods gives a significant sample of the *gambiae* population.

Comparison of these results also establishes the fact that *gambiae* feeds readily in the open and does not need to enter houses. This is a facet of behavior which must be investigated further in view of the fact that malaria-control spray programs are planned on the assumption that *gambiae* is endophilic.

PLASMODIUM INFECTION IN *A. GAMBIAE*

Three kinds of rates of natural infection are obtainable through dissection of wild-caught mosquitoes: the sporozoite rate, which is the percentage of mosquitoes dissected having sporozoites in the salivary glands; the oöcyst rate, or the percentage of mosquitoes dissected having oöcysts in the midguts; the index of

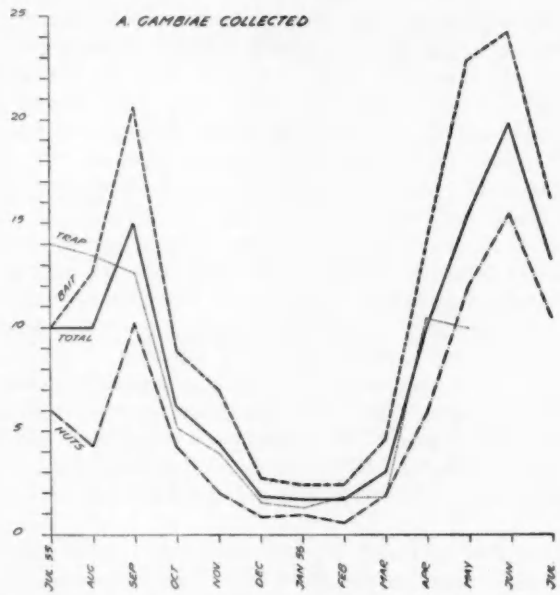

FIG. 3. Comparison of monthly catch per man night of *A. gambiae* by each of three collecting techniques.

natural infection, or the percentage of mosquitoes dissected having either sporozoites in the glands or oöcysts in the midguts (Covell *et al.*, 1953). Each of these rates is useful for particular purposes and each uncovers information not revealed by the other methods.

The sporozoite rate indicates the proportion of mosquitoes in the wild population that have reached an infective state and whose bite could transmit malaria at the time of capture. The dissection is made within hours after capture and can be performed on newly dead mosquitoes such as those collected in native houses by using a spray, as well as on mosquitoes collected alive.

The oöcyst rate indicates the proportion of mosquitoes in the wild population that have fed on persons with infective gametocytes in the blood. All these mosquitoes are potentially and in due course capable of transmitting malaria back to the human population. Mosquitoes with undigested blood in the midgut can not be dissected conveniently, and consequently the insects must be held alive for about three days before dissection. Aside from calculating a ratio, it is possible to obtain some useful corollary information from midgut dissections not available from gland dissections. The salivary gland either contains sporozoites or it does not; once they have reached the glands, the sporozoites undergo no further development. On the other hand, the oöcysts constantly undergo change as they develop, so that it is possible, within limits of course, to calculate the approximate age of the infection and thus to estimate when the infective feed oc-

curred. On the expectancy that *gambiae* feeds every fourth day (Muirhead-Thomson, 1951), it is possible to determine whether the infection was acquired on the night of capture, on the feed previous to that (about four nights before capture) or on the still previous feed (about eight nights before capture). Furthermore, a certain number of the midguts will have oöcysts young enough to enable identification of the species of *Plasmodium* involved.

To obtain an index of natural infection for a given anopheline population it is necessary to dissect both the midgut and the salivary glands in order to arrive at the proportion of mosquitoes having *Plasmodium* infections at any stage. Because the midgut dissection enters into the procedure the mosquitoes must be held for about three days to permit digestion to be completed, and to that extent the sporozoite rate extracted as part of the result may be diminished in value as compared to such a rate based on immediate dissection. Corollary information obtained from the oöcyst rate alone is available as part of this procedure and the value of such data may more than offset the disadvantages of delaying the gland dissection. Shute and Maryon (1952) plead for a wider use of midgut dissection by field workers and believe that the information obtained from dissecting both glands and midguts is far more accurate and useful than the data obtained from either alone.

From July 13, 1955 until July 24, 1956 the *gambiae* collected at the stations under consideration were held for three days, then were dissected for oöcysts. Of 12,322 *gambiae* collected during that period, 7,393 or 59.9 per cent were dissected, the residual 40 per cent representing mortality during the holding period. Beginning January 11, 1956 dissection of the salivary glands was added to the routine. From this point until the end of the study 6,632 *gambiae* were collected, and the midguts of 4,085 (61.6%) and the salivary glands of 3,605 (54.4%) were dissected.

The weekly results of this work are shown in Figure 4. From these data it is immediately evident that in this area infected mosquitoes are present at all times and that transmission never ceases. Assuming that the rate of infection in the mosquitoes is an index of transmission, transmission has an annual cycle. From Figure 4 it appears that during the year studied there were three peaks of infectivity separated by periods when the infectivity rates were somewhat lower: at the end of the rains from the beginning of October to late in November; in the middle of the dry season during February; at the beginning of the rainy season from the middle of May into the beginning of July.

Three factors influence the number of infected mosquitoes in a given area: the number and proportion of the human population having infective *Plasmodium* gametocytes at a given time; the size and age composition of the vector population; and the probability that any given mosquito will be able to take a human blood meal.

As to the last point, there is nothing to prevent the local *gambiae* population from regular feeding on the human population. Insecticide spraying is not used in the area studied and the construction of the native habitations allows completely free ingress and egress to mosquitoes. Bed netting is used by only the

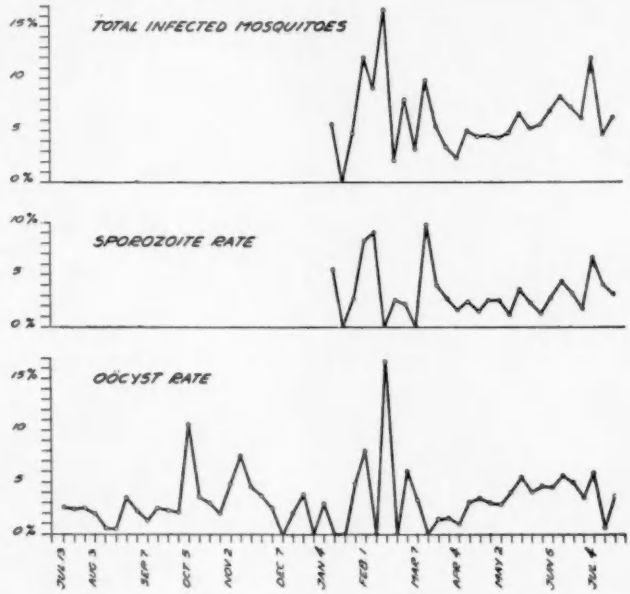

FIG. 4. Comparison of weekly oöcyst rates, sporozoite rates and rates of total infected *A. gambiae*.

very few people who can afford the equipment. Finally, the local population spends a surprisingly large number of hours after sunset in the open and our large catches by the night-collecting technique indicate that *gambiae* feeds readily out of doors.

A rough estimate of the size and age composition of the *gambiae* population may be made from the number of *gambiae* captured each week per collector-night. The influence of season and weather on the mosquito population has been discussed above. The *gambiae* population is much higher during the rainy season when there is a great abundance of suitable breeding water coinciding with favorably lower temperatures. During the dry months breeding water is reduced, though always present, and temperatures are higher, so that the *gambiae* population maintains itself at a much lower level. Prolonged periods of steady rain tend to encourage *gambiae* production, but sudden heavy torrents tend to flush out breeding sites and probably destroy part of the adult population.

During each rainy season there appears to be a regular sequence of events. At the end of the dry season showers become more and more frequent. The beginning of the rainy season is marked by frequent electrical storms, and soon the showers are replaced by deluges which often continue unabated for many hours, even days, and occasionally include periods of torrential rainfall when the rate may exceed an inch an hour. After several weeks to several months, the rain slackens and finally is reduced to a level similar to that of the dry season, a pe-

riod known locally as the "middle drys" which may last from a week to a month, but in any event is only a temporary reprieve. The rains then return in earnest for a month to six weeks, again including the occasional heavy torrent, before tapering off through a period of progressively lighter and shorter showers into the dry season. I have refrained from naming the months in which the stages of the rainy season occur, because there is a great deal of variation from year to year in the timing of the cycle. The "middle drys" occur almost always in August, but sometimes in late July. The rainy season may begin as early as late April or as late as the end of May; it may end as early as the beginning of October or as late as mid-December. But whatever the exact dates involved, the pattern is maintained.

The age composition of the *gambiae* population is directly affected by the rate of new emergences. High peaks of population represent periods when young mosquitoes predominate. At such times the ratio of infected mosquitoes goes down because so many of the adult females have not had the opportunity to acquire and incubate a malaria infection. Low periods of population are times when new emergences are reduced and the age composition then shifts to a greater proportion of older mosquitoes; the ratio of infected mosquitoes is then high.

All of this raises a question as to the real significance of the infection peak found in Figure 4 for February. For example the graphed peak for the week of February 15 is based on two infected mosquitoes out of only 12 dissections made at a time when each collector was capturing but a single *gambiae* a night. With the mosquito population so low, is the rate of infection really significant? Do two infected mosquitoes out of twelve, a rate of 16.7 per cent, actually represent a more serious threat than the 23 infected mosquitoes out of 502, a rate of 4.6 per cent, found during the week of May 30?

Perhaps a truer epidemiologic evaluation can be reached by taking into consideration both the rate of infection and the population size. One way of doing this is to multiply the rate of infection by the catch per man-night. Figure 5 is a week by week graph of the *gambiae* catch per man-night (Fig. 1) multiplied by the weekly oöcyst rate, the only rate calculated for the full year (Fig. 4). This method of computation places the most important season of malaria trans-

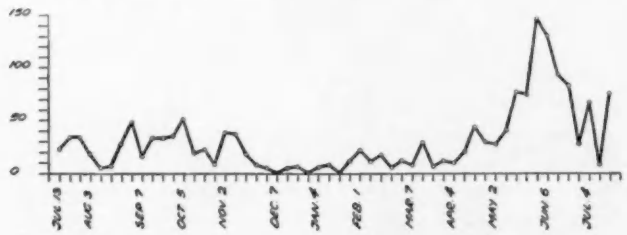

FIG. 5. Graphic analysis of the seasonal variation of malaria infection in *A. gambiae*, taking into consideration both the rate of infection and the size of the mosquito population. Weekly values are on an arbitrary scale representing the product of the oöcyst rate and the catch per man night.

mission, as calculated from infection rates in *gambiae*, in the period from the middle of April through July, with a second important period from the end of the "middle drys" in August until mid-November. The February peak, so prominent when mosquito infection rates alone are considered, becomes in Figure 5 merely a flurry of anticipation for the period of heavy transmission to come a few months later.

The obverse view lies in an intangible factor involving attitudes in the human population. The people living in this area are not really concerned with mosquitoes as malaria vectors, a concept they readily accept but almost certainly do not visualize. Their real objection to mosquitoes is the practical view that they are an annoyance which, when they are present in numbers, interferes with sleep. A few mosquitoes are not particularly annoying. Thus when mosquito density is low, the few infected mosquitoes present have perhaps an enhanced opportunity to effect transmission.

Horsfall (1953) summarizes records from literature on the incidence of *Plasmodium* in wild-caught *gambiae*. Considering only those records giving both gland and midgut dissections, the reported 19,275 midgut dissections included 2,326 positives, a rate of 12.0 per cent, and 20,090 salivary gland dissections gave 1,567 positives, a rate of 7.8 per cent. In the present study from January 11 to July 24, 1956, 158 of 4,085 midguts, or 3.9 per cent, were positive, and 105 of 3,605 salivary glands, or 2.9 per cent, were positive. To those who have not given the matter much thought the higher incidence of midgut infections over gland infections may come as a surprise. The reason lies in the duration of the cycle of development of the parasite in the mosquito which exceeds the life-span of a certain proportion of the wild mosquito population.

P. falciparum is the common type of malaria here. Its development in *A. gambiae* has been followed in a number of experiments in our laboratory under conditions of temperature and humidity closely approximating those characteristic of this area. From these experimental feedings it was found that oöcysts first are seen on the fourth day after the infective feeding and that they persist, thanks to the few which lag in development, up to at least the fifteenth day; sporozoites have appeared in the glands as early as the eighth day after the infective feeding, but the tenth day is the average here. Mayne (1919) found active sporozoites of *falciparum* in an *A. punctipennis* 68 days after infection. While the longevity of sporozoites in *A. gambiae* has not yet been tested under local conditions, there is reason to believe that sporozoites may be present in the glands for at least thirty days provided the mosquito lives long enough.

The difference between sporozoite and oöcyst rates affords data for estimating the average age of the local mosquito population. Boyd (1930) compared the sporozoite rate with the total infection rate (sporozoites and oöcysts) for the purpose. It is equally feasible to compare the sporozoite rate with the oocyst rate, as illustrated in Figure 6. With an oöcyst rate of 3.9 per cent and a sporozoite rate of 2.9 per cent, the indication is that the average mosquito's life is terminated when the sporozoites have been present in the glands for a period of time equal to three quarters of the period when the oöcysts were present. If the

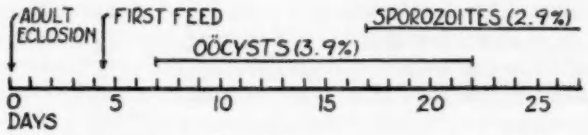

FIG. 6. Illustration of a method of estimating average age of the wild *A. gambiae* population from the ratio between the oöcyst rate and the sporozoite rate.

infection were acquired at the first feeding, when the mosquito was four days old as an adult, this places the average life span of the mosquitoes dissected at 27 days.

In our laboratory colony we find that the average female mosquito lives about 25 days as an adult. Muirhead-Thomson (1952) suggests that the natural life of the mosquito is much longer and that the sugar water usually fed as a supplemental diet in the laboratory reduces the life span. The calculations of longevity above indicate that there is no great discrepancy in the life span of the colony mosquito in our laboratory as compared to the local wild population.

Returning to Figure 4, it is noted that the peaks and valleys of the graph for the oöcyst rate precede peaks and valleys of the sporozoite rate graph by about a week, while the graph for the rate of total infected mosquitoes combines the fluctuations of both. Basically, any of these three graphs yields the same information, with greater accuracy inherent in the rate of total infected mosquitoes. Both midgut and gland dissections indicate the same periods of high or low transmission. Controversies on the relative merits of the two methods used for this purpose would seem to be more philosophic than real. For most workers the much more compact and easily interpreted graph developed from data on the total infected mosquitoes would not be justified by the greatly increased effort needed to develop it.

The field worker will select the method best yielding him the information he wants. If he is a member of a malaria control team and his purpose in studying the mosquitoes is to assess the progressive value of control measures, no doubt he will spray-catch mosquitoes resting in houses and will immediately dissect the salivary glands to obtain the sporozoite rate. Both the collecting technique and the laboratory follow-up are simple and direct, involving minimum expenditure of time and money. The weekly or monthly comparison of rates obtained in controlled areas with rates obtained in uncontrolled natural areas would give an adequate estimate of team progress. In brief, the sporozoite rate shows the potential influence of the mosquito population at a given time upon the human population.

The oöcyst rate yields basically the reverse information, that is, the influence at a given time of the human population upon the mosquito population. Yet it must be remembered that the mosquitoes having oöcysts today will have infective sporozoites next week, give or take a few days, and to that extent the oöcyst rate is the more significant and enables an earlier prediction of the malaria problem of the very immediate future. The additional information obtainable

TABLE 2

Comparison of infectivity rates obtained separately from mosquitoes collected by two methods

	Collected from huts	Collected outside
No. midguts dissected	1017	1513
No. midguts positive	47	64
Per cent midguts positive	4.62	4.23
No. glands dissected	939	1378
No. glands positive	31	39
Per cent glands positive	3.30	2.83
No. positive both glands and midguts	9	5
Total infected mosquitoes	69	98
Per cent infected mosquitoes	7.05	6.80

from dissecting the midguts would not, as a rule, be of any particular interest to the malaria control team.

VALIDITY OF COLLECTING TECHNIQUES FOR SAMPLING INFECTIVITY

From May 18 to the end of the study the *gambiae* taken by each of the three collecting methods were recorded separately when dissected in an effort to ascertain whether or not one or another method might be preferable for determining infectivity rates. The results of this study appear in Table 2. Application of the X^2 test for homogeneity of the two sets of samples (Simpson and Roe, 1939: pp. 303, 304) demonstrates that either method of collecting gives substantially the same results for the purpose and that the night-collecting procedure produces just as valid a sample as does the more usual hut-collecting. Not only do *gambiae* feed readily in the open, but those feeding in the open include the same proportion of older and infected mosquitoes as were found resting in the huts. This supports our belief (Fox and Fox, 1955) that *A. gambiae* tends to accept the first available blood meal when the time for feeding arrives.

The man-baited trap was used so little after May 18 at the collecting sites under consideration that the numbers concerned are too small to be included in Table 3.

IDENTIFICATION OF *PLASMODIUM* SPECIES FROM OÖCYSTS

Midgut dissections in surveys have a useful advantage in that a certain number of oöcysts will be small enough to afford identification of the *Plasmodium* involved. Furthermore, the oöcyst density may be of interest. Beginning July 25, 1955 the number of oöcysts in each positive midgut was recorded, along with the diameter of each cyst. Beginning September 24, 1955 all oöcysts were studied for identification characteristics and where possible the species was recorded.

Shute and Maryon (1952) point out that "all species of human malaria oöcysts, from the youngest stage to beyond the half-grown stage, can be diagnosed with some degree of certainty. This is even so when there are mixed infections in the

same mosquito." These authors describe in detail the characteristics of the oöcysts permitting specific identification of the species infecting man. The discussion by Brumpt (1949) and the photographs in Boyd (1949) are useful, and further information on particular species is to be found in the studies of Eyles and Jeffery (1949) and Jeffery (1952). In general, the *Plasmodium* species infecting man can be readily differentiated in nearly every case where the oöcyst is no larger than 25 μ in diameter, and sometimes the typical specific pigment pattern is retained until the oöcyst is nearly 35 μ in diameter.

Midgut dissections were made in saline to which mercurochrome had been added (Eyles, 1950). The dye greatly simplifies the recognition of very young oöcysts, which are otherwise difficult to locate and doubtless often missed. After dissection the midgut was transferred to an ample drop of clear saline and covered with a 12 mm. #2 round cover slip, which is light enough to float on top of the drop of saline. Then, while under continuous observation under low magnification, excess saline was withdrawn with a strip of paper towelling, taking great care to avoid flattening any oöcysts which might be present. The midgut was then searched for oöcysts under a magnification of 250 × (12.5 × ocular with 20 × objective). Any found were measured with a calibrated ocular micrometer under a magnification of 594 × (12.5 × ocular with 47.5 × objective). Oöcysts retaining characteristic pigment were studied and, where possible, identified. Identification is sometimes made easier by withdrawing enough more of the saline from the preparation to cause some flattening of the oöcysts, but care must be taken to avoid causing them to burst. Table 3 summarizes the monthly oöcyst densities and the specific identifications made.

It is of interest that higher oöcyst densities for October and November and

TABLE 3

Monthly density of oöcysts in positive midguts and identification of Plasmodium *species*

Month	Number positive midguts	Total number oöcysts	Number oöcysts per gut	Single infections			Mixed infections		Mosquitoes with identifiable infections	
				falciparum	*malariae*	*ovale*	*falciparum* and *malariae*	*falciparum* and *ovale*	number	per cent
September	8	20	2.5	5	0	0	0	0	5	62.5
October	17	74	4.5	5	0	1	0	2	8	47.0
November	19	84	4.4	6	1	2	1	0	10	52.6
December	3	4	1.3	0	1	0	0	0	1	33.3
January	3	9	3.0	1	0	0	0	0	1	33.3
February	4	5	1.3	2	0	0	0	0	2	50.0
March	7	8	1.1	1	0	0	0	0	1	14.3
April	17	78	4.6	4	0	0	0	1	5	29.4
May	46	250	5.4	11	2	0	0	0	13	27.2
June	64	670	11.5	16	0	0	0	0	16	25.0
July	18	105	5.8	1	1	0	0	0	2	11.1
Totals	206	1307	6.4	52	5	3	1	3	64	31.1

for May, June and July confirm the two peaks of transmission already indicated on the basis of oöcyst and sporozoite rates. The fact that the apparent peak in February does not also involve an increase in oöcyst density further suggests that rate alone may give a false evaluation of the epidemiologic situation.

During the eleven-month survey of species one third of the infected midguts contained identifiable oöcysts. Since it is usual for some of the oöcysts to lag behind in development, heavier infections having on the whole well-developed oöcysts frequently included a few still retaining clear pigment characteristics admitting identification. This accounts for the fact that one third of the infected midguts could be identified, which was rather more than was expected.

Since each mosquito with identifiable double infections represents two separate identifications of species, it can be seen from Table 2 that 68 identifications from oöcysts were made in 64 mosquitoes. Of these 56 (82.4%) were *falciparum*, 6 (8.8%) were *malariae* and 6 (8.8%) *ovale*. Four double infections were identified in which *falciparum* was associated once with *malariae* and three times with *ovale*.

The double infection of *falciparum* with *malariae* had two oöcysts of *malariae* on the gut wall, 30 and 32 μ in diameter respectively, and six *falciparum* oöcysts ranging from 15 to 22 μ in diameter. Data being accumulated in our laboratory on oöcyst growth rates under local conditions indicate that at the time of dissection the *falciparum* infection in this mosquito was five and a half days old, the *malariae* infection eight and a half days old. Thus they must have been acquired by the mosquito on two separate feedings. A mixed infection of *malariae* and *falciparum* acquired at a single feeding is not likely to produce identifiable oöcysts of both species at the same time, except for a very brief span, and it is even more unlikely that such a mosquito would be collected at just the right time to be dissected during that span.

Not included in the data but interesting to note in passing is the fact that even when oöcysts are too far advanced to be identified, the existence of a successive double infection can sometimes be recognized by the fact that there are two groups of oöcysts present at distinctly separate stages of development. These can be distinguished from single infections with lagging oöcysts by the fact that laggards are normally a small minority and include individual oöcysts which collectively grade in size up to the majority. Sometimes, too, a mosquito with sporozoites in the salivary glands will also have young oöcysts in the midgut; this situation indicates successive infections.

Although *P. vivax* from an imported strain has been studied here in experimental mosquito infections and the young oöcysts seem distinctive and easily distinguished from the other three species infecting man, no *vivax* was found in the wild-caught *A. gambiae* in this area.

DISTRIBUTION OF OÖCYSTS IN THE *A. GAMBIAE* MIDGUT

A curious but minor aspect of the present study of oöcysts is the fact that in *A. gambiae* oöcysts apparently do not conform to the distributional pattern observed in other anophelines. Previous observers have been almost unanimous

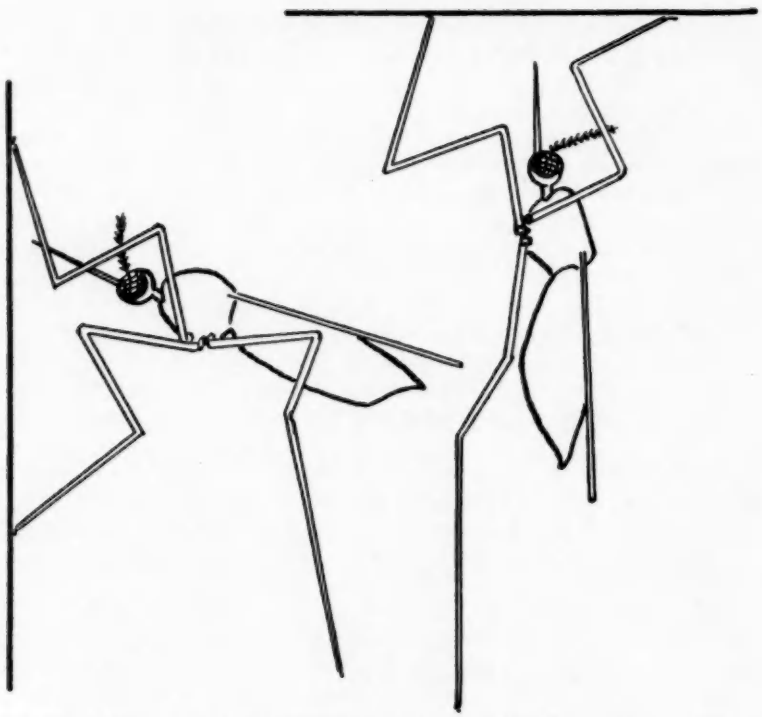

FIG. 7. Resting positions of *A. gambiae* on horizontal and on vertical surfaces

in stating that oöcysts tend to be placed in the lower or in the lower and middle portions of the midgut with very few or none to be found in the upper part. Huff (1934; 1941), dealing with a similar phenomenon in *Culex* and bird malaria, explained it as the consequence of the resting position of the mosquito. Shute (1938), working with *A. maculipennis atroparvus*, and Shute and Maryon (1953), dealing with *P. vivax* in *A. m. atroparvus* and *A. stephensi*, found that in the former oöcysts are located only on the lower half or two-thirds of the midgut, even when extremely dense, while in the latter mosquito the oöcysts may be distributed throughout the midgut but are very much denser in the lower half.

From the middle of May to the end of the study a record was kept of the distribution in 124 midguts containing 965 oöcysts. For the purpose the midgut was divided into an upper, a middle and a lower third. It was found that 349 oöcysts (36.2%) were located on the upper third, 439 (45.5%) on the middle third and only 177 (18.3%) on the lower third. Thirty nine of these midguts each had but a single oöcyst present: in 11 of them (28.2%) the oöcyst was in the upper third, in 24 (61.5%) it was on the middle third and in 4 (10.3%) in the lower third. In the nine midguts having more than 25 oöcysts each the distribution was as follows: 187 oöcysts (39.3%) were in the upper third, 233 (47.7%) in the middle third and 68 (13.0%) in the lower third.

Shute and Maryon (1953) explained the difference in oöcyst distribution between *A. m. atroparvus* and *A. stephensi* by pointing out that the salivary secretion of *atroparvus* rapidly agglutinates the ingested blood, whereas in *stephensi* the ingested blood is slowly coagulated. An explanation along such a line, that is, agglutination or coagulation, probably accounts for the distribution in *gambiae*. Certainly the resting position of *gambiae* cannot explain the high number of oöcysts in the anterior third of the midgut as *gambiae* always rests with the anterior end higher than the posterior end, even when clinging to a vertical surface, as shown in Figure 7.

NATURAL FILARIAL INFECTIONS IN *A. GAMBIAE*

In the only previous work on the mosquito vectors of bancroftian filariae in this area, Gelfand (1955a) showed that *A. gambiae* and *A. melas* are the most important. He found no mature (third stage) filariae in two other anopheline species and eleven other culicine species surveyed. There has been no previous study of seasonal incidence of filariae here, either in the patient or in the vector, and apparently the belief is that none is to be expected.

From October 1955 through July 1956 all wild caught *gambiae* were dissected for filariae. Only third stage worms were counted; measurements were taken with a calibrated ocular micrometer to verify the stage of development. While specific identification is not feasible from stages found in the mosquito, it is assumed that these were *Wuchereria bancrofti*.

A month by month summary of the data appears in Table 4. The overall rate obtained was 3.6 per cent based on 5,566 dissections. This is rather higher than the rate of 1.5 per cent obtained by Gelfand (1955a) from *A. gambiae* in approximately the same area, but the greater number of dissections made in the present study and the greater regularity of the work probably account for the difference. Gelfand dissected 262 wild caught *gambiae* during a sixteen month period.

The seasonal variation is interesting and seems to suggest that a regular cycle exists in the rate of infection to be found in the vector, but a cycle dissimilar to

TABLE 4

Monthly rate of filarial infection in wild caught A. gambiae

Month	Number dissected	Number positive	Per cent positive	Total no. filariae	Filariae per positive
October	520	6	1.15	29	4.8
November	387	5	1.29	17	3.4
December	146	4	2.73	26	6.5
January	86	3	3.48	4	1.3
February	112	2	1.78	13	6.5
March	292	8	2.73	17	2.1
April	769	19	2.47	60	3.2
May	1437	56	3.89	377	6.7
June	1292	71	5.49	492	6.9
July	525	26	4.95	195	7.5
Total	5566	200	3.59	1225	6.1

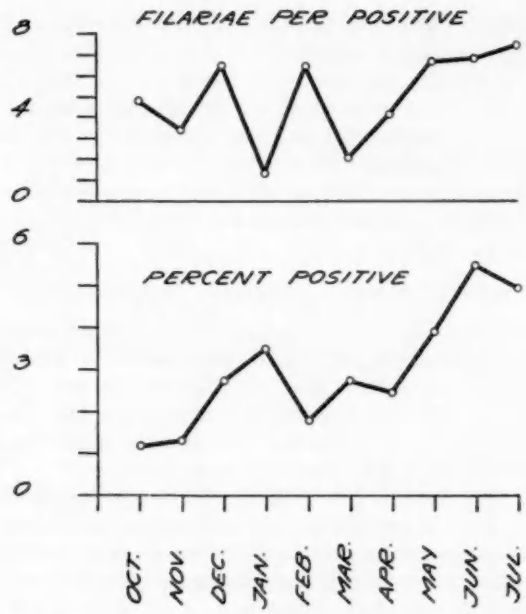

FIG. 8. Monthly rate of filarial infection found in *A. gambiae*

chat found for rates of malaria infection. Comparing the data on filarial infections in *A. gambiae* in Figure 8 with the data on the population density of *gambiae* in Figure 2, it is noted that the rate of infection rises and falls directly with the population. During the dry months when the *gambiae* population is low, the rate of filarial infection also is low. With the increase in mosquito population at the beginning of the rains, the rate of infection also rises. Not only does the rate of infected mosquitoes thus increase, but at the same time the number of third stage filariae per infected mosquito also rises. It would appear that the period of greatest transmission is during May, June and July for the period studied.

Earlier it was shown that *gambiae* collected on human bait in the open give significantly the same rate of infection with malaria as do the mosquitoes collected from houses. In Table 5 similar data are given to compare the value of the three collecting methods in assessing filarial infections in the wild mosquito population. From this table it may be noted that there is no significant difference in the percentage of mosquitoes with filarial infections found among mosquitoes collected on human bait in the open and those found resting in the huts. Apparently any of the three methods yields a valid sample.

In addition to the collecting stations used for data on the malaria infection rates, Snafu village was included as a source of mosquitoes from which to estimate the filaria rates. Snafu village is located on the north bank of the Gboe river (called the Du in previously published studies, but properly the Du river

TABLE 5

Comparative value of three methods of collecting for filarial rates

	Collected from huts	Collected at bait	Collected in trap	Total of all methods
Number dissected	975	1543	93	2611
Number positive	51	76	6	133
Per cent positive	5.23	4.92	6.45	5.09
Total no. filariae	287	659	50	996
Average filariae per positive	5.6	8.7	8.2	7.5

is a tributary flowing through the Firestone plantation and joining the Gboe some miles upstream from Snafu). During the dry season a certain number of *A. melas* are to be found at Snafu, but during the rainy months this species is absent or extremely rare there, according to egg identifications made. Mosquitoes from Snafu were included in the filaria data only during the wet months and it is believed that *melas* has not entered significantly into the picture.

Table 6 shows the total filaria infection rates for each of the collecting stations. The rates found have been noted on the map (Figure 1) and an interesting geographic situation is observed; the point of greatest infection of those studied is Snafu village on the Gboe river. Proceeding inland from the river the rate of infection becomes progressively lower. Similarly, on Far creek, Porblah village near the Farmington river has an incidence of filaria infection more than twice that of Farwein, located well up the creek. At all stations transmission occurs throughout the year but is greater during the rains.

This geographic pattern may be, on a very small scale, part of the broader phenomenon which Poindexter (1950) noted in a Liberia-wide survey, namely, that the clinical incidence of filariasis is greatest near the coast and diminishes as one goes inland until, in some upcountry areas, almost no positive cases are

TABLE 6

Filarial infection rates in A. gambiae *from villages collected*

Village	Number dissected	Number positive	Per cent positive	Number of filariae	Filariae per pos.	Infection index*
Beercase	415	18	4.33	73	4.1	17.8
December	884	29	3.28	135	4.7	15.4
Farwein	236	5	2.11	31	6.2	13.1
Gblogblo	109	2	1.83	9	4.5	8.2
Jallah	297	6	2.02	52	8.7	17.6
Musah	752	19	2.52	117	6.2	15.6
Peabody	144	2	1.38	11	5.5	7.6
Porblah	344	16	4.65	74	4.6	21.4
Sammy	1314	62	4.71	399	6.4	30.1
Snafu	432	29	6.71	244	8.4	56.4
Tar	393	12	3.05	61	5.1	15.6

* Infection index: an arbitrary figure reached as the product of the percentage of positive mosquitoes and the number of filariae per positive mosquito.

to be found. Poindexter (1950) also presented evidence to support the importance of repeated exposures to the bite of infected mosquitoes in producing clinical filariasis. Accordingly, higher clinical rates should be expected from villages near the large coastal rivers, lower rates from villages situated back from the rivers and on higher ground.

Gelfand (1955a) found that salt-marsh breeding *A. melas* had a rate of filarial infection more than twice that of fresh water *gambiae*. Burch and Greenville (1955) state that since *melas* apparently is a more efficient vector than *gambiae*, this might help to account for the country-wide geographic pattern of clinical filariasis found by Poindexter which their findings confirmed.

But since Gelfand's data were based on 262 *gambiae*, 306 *melas* and 592 unidentifiable mixed *gambiae-melas*, there remains some doubt that *melas* actually is so much more efficient as a vector of filariae than *gambiae*. The rate of third stage infections found in 5,566 *gambiae* in the present study of 3.6 per cent, which is close to the rate of 3.9 per cent that Gelfand found for *melas*, and is much greater than the rate of 1.5 per cent he found for *gambiae*. Possibly when a sufficient number of *melas* are dissected the rates will be found to be much the same for the two species.

Another though minor source of discrepancy lies in the fact that Gelfand discarded the abdomen, dissecting only the thorax and proboscis. As shown in Table 7, 12 or 6.0% of the infected mosquitoes were found to have third-stage filariae present in the abdomen only, and these would have been missed had the abdomens been discarded. Searching the abdomen for filariae also affects the analysis of density distribution, as Table 7 shows; of 1,231 third-stage filariae counted, 466 (37.9%) were found in the abdomen, decreasing somewhat the proportion of low density infections and increasing the higher densities, but by no means reaching the proportions in higher densities that Gelfand observed in *melas*.

TABLE 7

Distribution of filarial infections of mosquitoes according to density

Total mosquitoes		No. and % positive mosquitoes with			
dissected	infected	1–5 larvae	6–10 larvae	11–20 larvae	21–30 larvae
5,566	201 (3.6%)	105 (52.2%)	79 (39.3%)	14 (7.0%)	3 (1.5%)

Location of filariae in the mosquito

Total filariae	Proboscis	Thorax	Abdomen
1,231	272 (22.1%)	493 (40.0%)	466 (37.9%)
Total infections	**Proboscis and thorax**		**Abdomen alone**
200	188 (94%)		12 (6%)

SUMMARY

From June 1, 1955 to July 24, 1956 *Anopheles gambiae* were collected in villages in Marshall territory, coastal Liberia, using three collecting techniques: hand-catching mosquitoes on human bait in the open, trapping with human bait, and hand-catching mosquitoes found resting in the houses. All three methods yield valid population samples for estimating population size, and for studying both malaria and filariasis infectivity rates, demonstrating that *gambiae* feeds readily in the open and does not need to enter houses. The *gambiae* population is greatest during the rainy season from May to October, reaching peaks at the beginning and at the end of the rains, which are also the two periods of highest transmission. The *gambiae* population thus varies inversely with the temperature, which is lowest during the rains.

The oöcyst, sporozoite and total infection rates were found to yield the same epidemiologic pattern, the last being the most accurate and the easiest to interpret. The relationship between the sporozoite rate and the oöcyst rate can be used to estimate the average age of wild caught mosquitoes when the timing of the developmental cycle of *Plasmodium* in the mosquito is also known. The average age thus derived for the mosquitoes studied was 27 days. Species identification of oöcysts was possible in a third of the positive midguts; of these *falciparum* made up 82.4 per cent, and *malariae* and *ovale* each 8.8 per cent. *Vivax* was not found in wild *gambiae*. The disposition of the oöcysts in the midgut differed from that described in *A. atroparvus* and *A. stephensi*; the density was greatest in the middle third (45.5%), somewhat less in the anterior third (36.2%), and least in the posterior third (18.3%).

The overall rate of infection in *A. gambiae* with third stage bancroftian filariae (3.6%) was much higher than rates found by previous workers here, but is probably nearer the truth since it is based on many more dissections. Previous reports that *A. melas* is a more efficient vector of filariae than *gambiae* were based on small samples and are not convincing. Seasonal variation both in rate and in density of infection was noted, both increasing with the increase in the *gambiae* population at the beginning of the rainy season, both being lowest during the dry season. Geographically, rates of infection were found to be highest near the tidal rivers. becoming progressively lower inland, away from these rivers, and on higher ground.

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RESISTANCE OF *PLASMODIUM MALARIAE* TO PYRIMETHAMINE (DARAPRIM)

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It was reported recently that two *Plasmodium malariae* infections relapsing after a single 25-milligram dose of pyrimethamine (Daraprim®), showed such a very poor response to a second dose of the drug, consisting of 50 milligrams, that the erythrocytic parasites were not cleared from the blood stream (Young, 1957). There was also a third case (# 1271) where the initial treatment of 100 milligrams, namely, 50 milligrams given on each of two consecutive days, did not eliminate the erythrocytic parasites. Additional evidence is now available on the parasite resistance to this drug and such evidence is the basis of the present report.

The patients were receiving therapeutic malaria for neurosyphilis. All were Negroes except Nos. 592 and 1267 who were white. The infection was transmitted by the transfusion of about 10 cc. of malarious blood. The recent linear passages of the strain are shown in Figure 1.

The resistance appeared in Patient No. 1271 (Fig. 2). This linear strain of parasites had not been previously exposed to pyrimethamine. The first treatment with pyrimethamine, consisting of 50 milligrams daily for two days, was on parasite day 18, following which the parasites were greatly reduced, but they began to increase on the tenth post-treatment day (parasite day 28). On parasite day 31, another 100-milligram regimen was begun. The parasites continued to increase and the fevers returned. The third 100-milligram treatment on day 38 resulted in a parasite decrease and cessation of fevers, but after the fourth treatment on days 52 and 53, the parasites again increased and the fevers reappeared. The fifth treatment was started on day 66 and consisted of 50 milligrams daily for three days. There was a temporary decrease in parasites and absence of fevers for about two weeks. A return of fever necessitated the termination of the infection by chloroquine, 1.5 grams given over a three-day period. The parasites responded rapidly and the blood stream was cleared on the fourth day. On day 93, just before chloroquine therapy was begun, the malaria was transmitted to Patients No. 1278 and 1273 by blood inoculation.

Patient No. 1278 developed parasites 10 days later. On each of parasite days 19 and 20, 50 milligrams of pyrimethamine were given. The parasites temporarily decreased and the fevers ceased only for a few days.

Beginning on parasite day 33 and for 12 weekly intervals thereafter, 50 milligrams daily for two consecutive days were given. The drug apparently had little effect as the malaria course appeared to follow the normal pattern of a primary attack, showing 13 fevers at fairly regular intervals and parasitemias within normal ranges. On parasite day 74, the parasitemia dropped below 1,000 per

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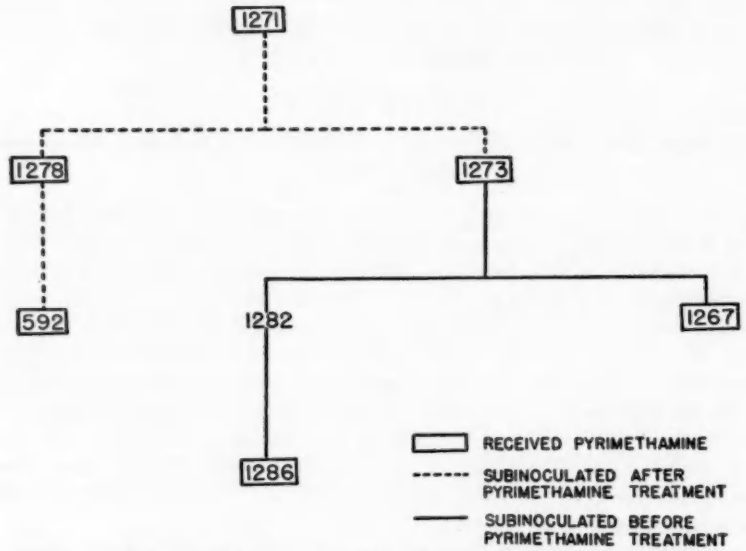

FIG. 1. A diagram of the passages of *P. malariae* after resistance developed.

cubic millimeter with a cessation of fevers. Although receiving weekly treatments the parasitemia exceeded 1,000 per cubic millimeter again on day 106 and a temperature of 101° F. occurred on day 109.

After 122 days of continuous parasitemia accompanied by 20 fevers, during which time 14 treatments of 100 milligrams each of pyrimethamine were given, the patient still had erythrocytic parasites. Chloroquine, 1.5 grams total in three days, was given and the parasites were eliminated rapidly.

Patient No. 592 was inoculated from Patient No. 1278 on parasite day 33. Pyrimethamine was begun after Patient No. 592 had experienced 19 fevers and 56 days of parasites. The response was slow, the first negative blood smear occurring after six weekly treatments of 100 milligrams. A relapse followed.

Patient No. 1273, who was inoculated at the same time as Patient No. 1278, did not receive pyrimethamine until parasite day 68, by which time 19 fevers had occurred. The parasite density decreased temporarily. A second 100-milligram course was started on day 104. Parasites were still present on day 193, at which time the infection was terminated with chloroquine.

Patient No. 1267 received malaria parasites from Patient No. 1273. Pyrimethamine, 50 milligrams given on days 44 and 45, resulted in a temporary clearance of parasites. On day 85, after the return of parasites and fevers, 25 milligrams had little effect. Chloroquine given on day 94 rapidly eliminated the parasites.

From Patient No. 1273, the strain was passaged to Patient No. 1282, who received no pyrimethamine, and from this patient to Patient No. 1286. As shown

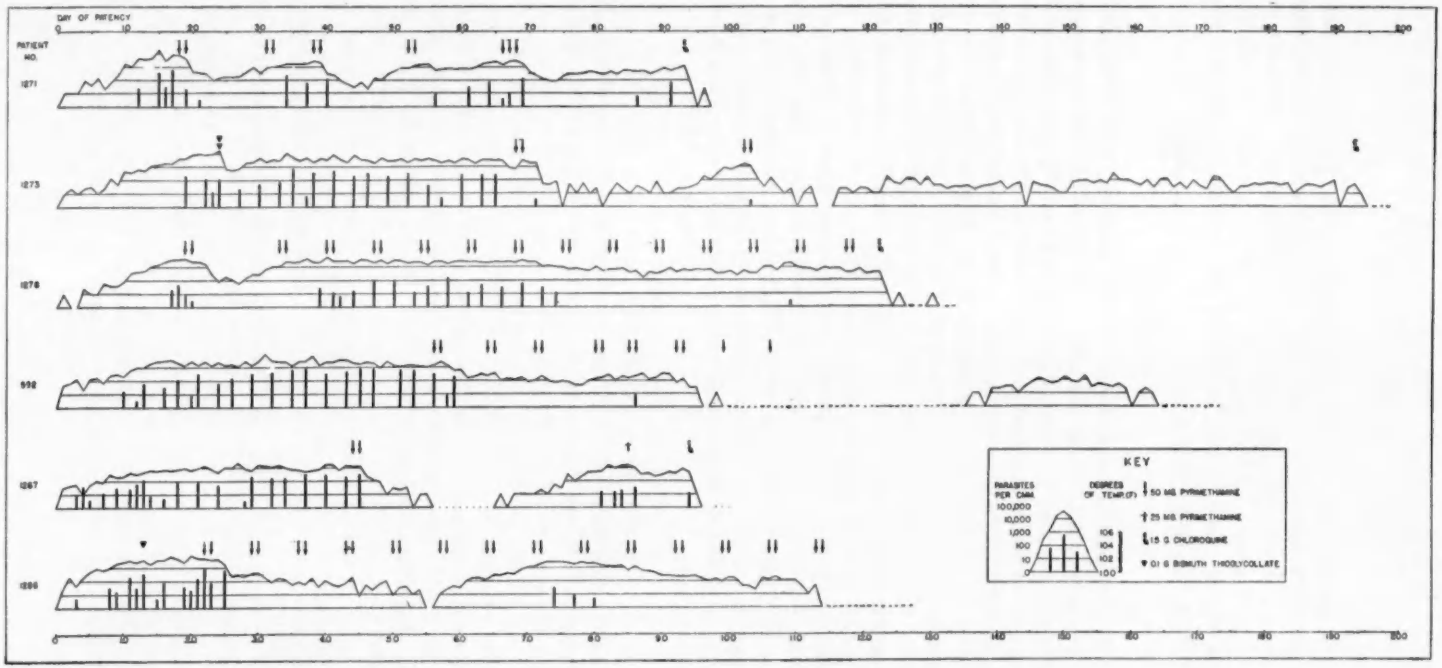

FIG. 2. The effect of pyrimethamine upon the parasitic and clinical activity of six *P. malariae* infections.

in Figure 2, weekly doses of 100 milligrams pyrimethamine given to the latter resulted in elimination of fevers for over two months. There was a temporary elimination of parasites but they returned to fever-producing levels on parasite day 74 during the eight week of drug administration. On day 114, following the fourteenth weekly dose of 100 milligrams pyrimethamine, the parasites disappeared from the blood smears.

DISCUSSION

Cases of *P. malariae* infections are presented in which resistance to pyrimethamine is demonstrated. The resistance appeared suddenly in a patient (1271) who first received the drug, 100 milligrams total in two days, during the third week of an induced primary attack. In 14 patients previously treated, from 25 to 100 milligrams of pyrimethamine had eliminated the erythrocytic parasites of this strain of malaria, although slowly (Young, 1957).

To date, six infections have shown varying degrees of resistance, usually of a high order. The most resistant case (1278) received 1,400 milligrams of pyrimethamine in a 14-week period during which time the patient experienced a fairly typical primary attack of malaria.

It has been suggested that resistance to pyrimethamine is likely to occur only under conditions where inadequate dosages, that is, less than 25 milligrams weekly, were given (Covell *et al.*, 1955). In the cases reported here, resistance has occurred in dosages four times the recommended weekly dose for suppression.

Urinalysis and simple blood studies were done. No reactions attributable to the drug were seen.

SUMMARY

Plasmodium malariae (USPHS Strain) has developed resistance to pyrimethamine (Daraprim®) where the amount of drug given was greater than that recommended for suppression.

ACKNOWLEDGMENT

Appreciation is expressed to Mr. Jimmie C. Skinner for technical aid, to the Staff of the South Carolina State Hospital for cooperation, and to Burroughs Wellcome Company, Tuckahoe, New York, for furnishing the pyrimethamine (Daraprim®).

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FURTHER STUDIES ON MALARIA SUPPRESSION BY MONTHLY DRUG ADMINISTRATION

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The excellent results obtained in malaria control in many parts of the world with residual-insecticide house spraying have not been realized in the intensely malarious areas of Tropical Africa. This is due to a number of factors among which the World Health Organization Expert Committee on Malaria (1956) mentions peculiarity in the habits of *Anopheles gambiae*, the prolonged period of the transmission season, the extremely high degree of endemicity of malaria in the region, restricted communications and the relatively low level of administrative development. To these may be added the custom of the people in some areas of living in temporary shelters during certain periods of the crop-growing season, and the exposure to mosquitos during frequent evening celebrations.

The appearance of resistance to insecticides in several species of anopheline vectors of malaria, reported from widely separated regions of the world, has emphasized the urgency of intensifying efforts aimed at malaria control and eradication. These developments focus attention on methods of control other than the use of imagocidal residual sprays and of these the role of antimalarial drugs must receive serious consideration.

Over 20 years ago Clark and Komp (1941) attempted to control malaria in Panama, in the absence of antimosquito measures, by monthly administration of quinine, and later Atebrin plus Plasmochin, to all inhabitants of an area who were found positive for malaria parasites. The experiment, which continued for 10 years, was not successful in controlling malaria.

With the development of more efficient antimalarials, interest in the use of drugs for malaria control was revived and a number of investigators have carried out limited tests on the effects of weekly suppressive doses of the 4-aminoquinolines and pyrimethamine in malaria control, with excellent results. However, mass drug administration at weekly intervals is impracticable. Pinotti (1954, 1955) in Brazil added chloroquine to cooking salt and reported a marked drop in malaria parasite rates when this salt was used exclusively, in rural endemic malarious areas. Further information on the efficiency and feasibility of this method of malaria control would be valuable.

Because the indigenous residents of Equatorial Africa show early development of a relatively effective resistance to malaria, it was considered possible that, for them, suppressive doses of antimalarials would be more efficient and act for longer periods of time than could be expected in nonresistant subjects. The studies of Schmidt and Genther (1953) on pyrimethamine and *Plasmodium cynomolgi*, which showed that the immune state of the host markedly influenced the efficiency of the drug in trophozoite-induced infections, reinforced this

opinion. If this were the case and the interval between treatments could be prolonged sufficiently, then malaria control by the use of drugs might be feasible. Accordingly, experiments testing the effects on malaria of pyrimethamine and chloroquine given once a month in suppressive doses, were started at this Institute several years ago. An earlier short-term study (Miller, 1955) with school children gave most promising results. The experiments of Delannoy and Hugon (1954) who reported on the effects of monthly pyrimethamine in children living in a highly malarious area in the Belgian Congo were also favorable. Their study was continued for six months and at the end of that period the parasite rate, originally 62 per cent, had dropped to zero. And Houel (1954), working in a meso-endemic malaria zone, showed that monthly pyrimethamine doses effected a marked reduction in the malaria incidence when tested over a four-month period. The present report gives the results of further experiments testing the effects of monthly suppressive doses of these antimalarials.

MATERIALS AND METHODS

The studies were carried out in the coastal area of Marshall Territory in Liberia. As mentioned in earlier reports (Miller, 1954; 1955) this region is intensely malarious with continuous transmission the year round. The predominant species of malaria parasite is *Plasmodium falciparum* although *P. malariae* is also common, especially in the younger age groups; *P. ovale* is seen sporadically; *P. vivax* is rare or absent. Recent investigations at this Institute by Bray (personal communication, 1956) indicate that in indigenous Liberians, parasites found in "stippled" erythrocytes are nearly all, if not all, *P. ovale*.

Four separate experiments, each in different population groups, are described here. In three, the drug given was pyrimethamine; in the fourth, pyrimethamine plus chloroquine was used. All drugs were given in suppressive doses at monthly intervals. Adults and children three years or over received pyrimethamine in 25 mgm. doses and chloroquine in 150 mgm. (base) doses; children under three years were given half doses or 12.5 mgm. pyrimethamine and 75 mgm. (base) chloroquine. Infants and very young children received the drug as a suspension in sugar and water. Children under three months were not included in the experimental groups.

The effects of chemotherapy were determined by malaria parasite-rate studies. Thick blood films were made from all subjects at monthly or weekly intervals depending on the experiment. Blood films were stained with Giemsa and studied by examining not less than 150 oil-immersion fields.

EXPERIMENT 1

Studies here were undertaken with women and children living in two labor camps of a nearby Plantation. These camps had received residual insecticide house-spraying at 4-month intervals with DDT for one year previous, but spraying was discontinued four months prior to the start of the experiment and the camps remained unsprayed for its duration and subsequently for several months.

Initially a house-to-house census was made in both camps and the names of the adult females, and names, approximate ages and sex of the children were re-

TABLE 1

Malaria parasite rates in two groups of adult females and children living in Plantation work camps, one group receiving monthly suppressive doses of pyrimethamine (25 mg.) and the other pyrimethamine (25 mg.) plus chloroquine (150 mg. base). Parasite rates for a control series in each group are also shown

Group	Months																	
	D	-1	-2	-3	-4	-5	-6	-7	-8	-9	-10	-11	-12	-13	-14	-15	-16	-17
Pyrimethamine																		
Number exam.	190	176	157	170	150	128	122	102	112	90	96	107	116	135	110	100	96	87
Number pos.	91	18	0	3	1	0	0	1	1	2	0	0	1	1	0	0	1	3
Per cent pos.	47	10	0	2	1	0	0	1	1	2	0	0	1	1	0	0	1	3
Control																		
Number exam.			33	13	22	15	22	41	32	38	44	46	43	29	26	45	47	41
Number pos.			12	7	9	2	4	10	7	8	7	10	13	4	5	14	9	8
Per cent pos.			36	54	41	13	18	24	22	22	15	22	30	14	19	31	19	20
Pyrimethamine plus chloroquine																		
Number exam.	240	196	160	166	175	162	140	153	144	117	108	135	122					
Number pos.	110	2	0	1	1	0	0	1	1	1	1	2	1					
Per cent pos.	46	2	0	1	1	0	0	1	1	1	1	1	1					
Control																		
Number exam.			36	36	37	28	49	38	53	43	96	35	52					
Number pos.			13	19	14	13	6	11	7	12	16	3	17					
Per cent pos.			36	53	38	45	12	29	13	28	17	8	33					

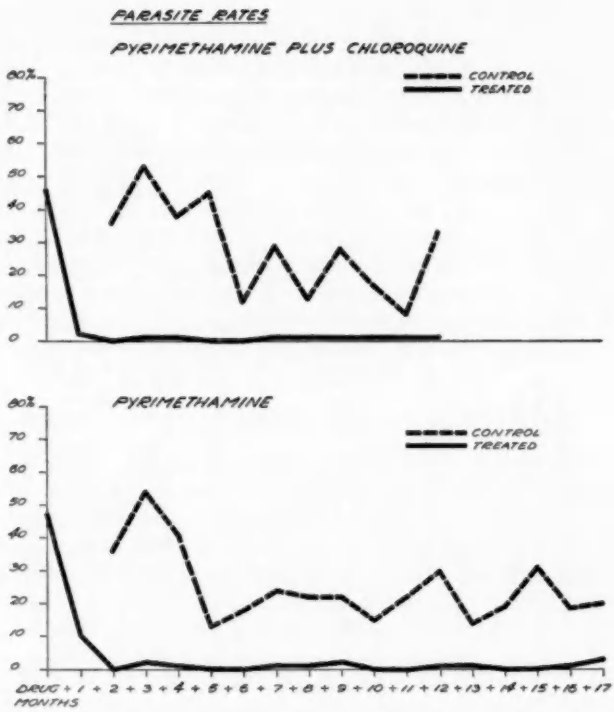

FIG. 1. Malaria parasite rates in two groups of adult females and children living in Plantation work camps, one group receiving monthly suppressive doses of pyrimethamine and the other pyrimethamine plus chloroquine. Parasite rates for a control series in each group are also shown.

corded. A record card was kept for the residents of each hut. Thus it was possible to maintain a treatment and blood-examination history for each individual. Children under 10 years comprised about one-third of the population studied.

In camp A the subjects received pyrimethamine at monthly intervals and at the same time a blood film was taken from each. Experimental subjects missing treatment the previous month were included with new-comers to the camp; all were examined for malaria parasites and constituted the 'control group'. Once they received treatment they were included with the 'treatment group' the following month. Throughout the course of the experiment the numbers treated and studied per month ranged from 90 to 176. The study continued for 18 months.

In Camp B the same procedure was adopted as that used for Camp A except that each subject received both pyrimethamine and chloroquine at monthly intervals. The numbers treated and studied per month ranged from 108 to 196 and the experiment continued for 13 months.

Results. Parasite rates for both groups are shown in Table 1 and Figure 1.

Because the numbers of women and children were small they are here treated together and the results represent the combined malaria parasite rates of all age groups.

In Camp A where pyrimethamine was given, the parasite rate fell from 47 per cent to zero by the second month of treatment and throughout the remaining 16 months never rose above 3 per cent. The mean monthly parasite rate for the entire treatment period was 0.7 per cent. The control group showed parasite rates ranging from 13 to 54 per cent and the mean monthly rate was 25 per cent.

Subjects in Camp B, receiving pyrimethamine plus chloroquine, showed an initial parasite rate of 46 per cent which fell to zero by the second month of treatment and never rose above 1 per cent throughout the treatment period. The mean monthly parasite rate for the treatment period was 0.6 per cent. The controls showed monthly malaria parasite rates ranging from 8 to 53 per cent with a mean monthly rate of 28 per cent.

This experiment showed that monthly suppressive doses of pyrimethamine alone or in combination with chloroquine, were very effective in reducing malaria parasite rates to a negligible level. Pyrimethamine was practically as efficient when used alone as when given in combination with chloroquine. It will be noted that for certain months the parasite rates in the control group were comparatively low, but it must be remembered that this group included a varying proportion of individuals who received treatment two or more months previously and would therefore show a lower rate than might be expected in a fully untreated group. Moreover, the anopheline density in this area was low and the malaria infection challenge considered to be of a relatively low order.

EXPERIMENT 2

This study comprised three groups of adult males who lived in different Plantation work-camps, all of which had received residual DDT house spraying at four-month intervals some time prior to the start of the experiment. In camps C and D spraying had been discontinued and there was no spraying for four months prior to or throughout the course of the experiment. All subjects in Camp C received 25 mgm. pyrimethamine once each month and constituted the treated group, while those in Camp D were untreated and served as controls. In the third camp, Camp E, the subjects were presumably protected by residual insecticide house spraying and no drugs were given. At monthly intervals blood films were taken at random from 100 individuals in each camp and the malaria parasite rates and crescent rates determined.

It is important to note that the subjects of this experiment were only partially controlled and no attempt was made to identify the individuals, of whom a not inconsiderable proportion migrate within and in-and-out of the Plantation area. Each group included a population which varied with the seasons from about 300 to 375. The study was continued for 24 months.

Results. The malaria parasite rates for the three groups are shown in Table 2 and Figure 2. In Camp C where subjects received drug treatment the parasite rate dropped from 40 to 5 per cent one month after the initial drug administration and ranged between 1 per cent and 14 per cent during the subsequent 23 months

TABLE 2

Malaria parasite and crescent rates in three groups of adults living in separate Plantation work camps, one group receiving monthly suppressive doses of pyrimethamine (25 mg.), the second protected by residual insecticide house spraying and the third an unprotected control group

Group	Months																							
	D	-1	-2	-3	-4	-5	-6	-7	-8	-9	-10	-11	-12	-13	-14	-15	-16	-17	-18	-19	-20	-21	-22	-23
Pyrimethamine																								
Paras. rate	40	5	1	6	5	14	3	5	2	2	7	10	7	2	2	3	4	5	7	9	4	4	3	4
Cresc. rate	6	1	1	0	0	1	0	0	0	1	0	1	2	0	1	0	1	0	0	0	0	0	1	1
Control																								
Paras. rate	25	27	26	27	12	23	28	21	22	31	37	41	34	30	34	17	23	30	21	39	37	27	29	23
Cresc. rate	0	1	1	5	0	1	5	4	0	1	4	1	2	1	7	3	2	5	1	3	0	1	1	1
Insecticide																								
Paras. rate	28	37	30	34	30	23	18	23	13	13	20	21	26	26	22	21	23	38	34	21	28	25	24	32
Cresc. rate	2	2	1	0	2	1	2	1	0	1	0	4	3	3	2	3	2	3	1	1	2	1	4	4

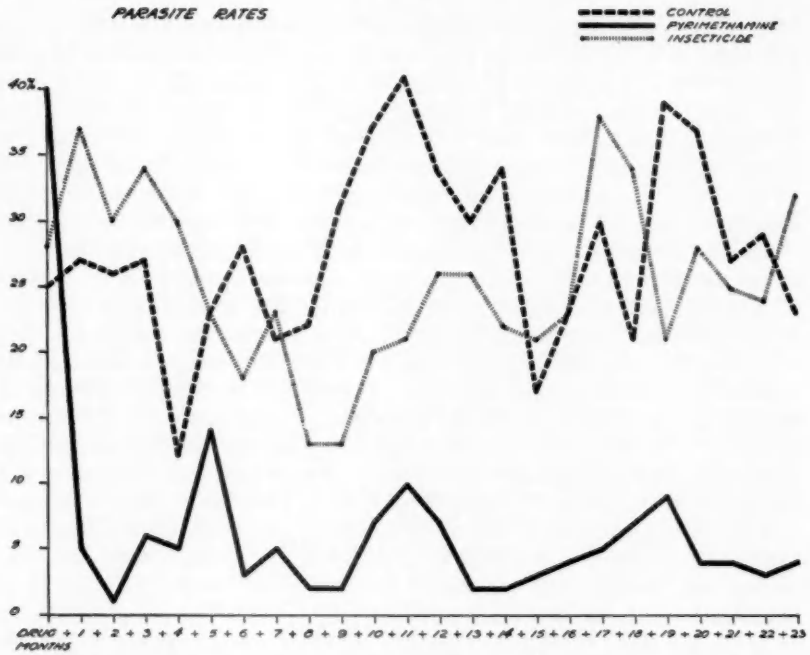
PARASITE RATES
CONTROL
PYRIMETHAMINE
INSECTICIDE
40%
35
30
25
20
15
10
5
0
DRUG + 1 + 2 + 3 + 4 + 5 + 6 + 7 + 8 + 9 + 10 + 11 + 12 + 13 + 14 + 15 + 16 + 17 + 18 + 19 + 20 + 21 + 22 + 23
MONTHS

FIG. 2. Malaria parasite and crescent rates in three groups of adults living in separate Plantation work camps, one group receiving monthly suppressive doses of pyrimethamine, the second protected by residual insecticide house spraying and the third, an unprotected control group.

of the study. The mean monthly parasite rate for the entire treatment period was 5 per cent and the mean monthly crescent rate was 0.5 per cent. The control subjects in Camp D, who were unprotected, showed parasite rates ranging from 12 to 41 per cent with a mean monthly rate of 28 per cent; the mean monthly crescent rate over the 24 months of the experiment was 2.2 per cent. The subjects in Camp E, presumably protected by residual insecticide spray, showed monthly parasite rates ranging from 13 to 38 per cent over the two years of study and the mean monthly parasite rate was 22 per cent. The mean monthly crescent rate in this group was 2.0 per cent.

The results indicate that even in a population including a certain migratory portion, under the conditions of our experiment relatively good results may be expected from the monthly administration of suppressive doses of pyrimethamine. This was in contrast to the apparently indifferent value of residual insecticide spraying for malaria control seen in this experiment.

EXPERIMENT 3

This experiment deals with a small group, 20 young male adults living in an unsprayed area, who had previously been studied intensively and continuously

for a period of one year (Miller, 1955a) during which time treatment for malaria was witheld. In this period the members of the group showed malaria parasites in 21 per cent and crescents in 6 per cent of all blood examinations. They suffered 32 attacks of clinical malaria which resulted in 130 days of morbidity. Following completion of the above study the members of the group were started on monthly suppressive doses of pyrimethamine, and routine blood films were taken weekly; when malaria parasites were found, the blood was examined daily until three consecutive daily examinations were negative for parasites.

Results. During the year of treatment seven of the 20 subjects showed *P. falciparum* parasites. In each of six subjects, parasites were seen only on one occasion even though blood examinations were made on the days following the appearance of the parasites. The seventh developed a clinical attack of malaria five days after the date of the fifth monthly drug dose. On the third day of the attack the patient received 25 mgm. of pyrimethamine and two days later the blood was clear of asexual malaria parasites; crescents appeared in the blood later and persisted for several weeks. The short period elapsing from the day the drug was supposedly taken to the onset of the attack, plus the fact that treatment with the same drug in the same dosage effected rapid clearance of asexual parasites from the blood, suggests the possibility that this patient may not have taken his treatment on the prescribed date prior to the attack. In any case, even if all subjects are included, asexual parasites were seen only in 0.1 per cent of all blood films examined, and if the clinical case is excluded, crescents never appeared in the blood of treated subjects. This was in contrast to the 21 per cent parasite rate and 6 per cent crescent rate shown by the same group the previous year when treatment was withheld. Finally, there was only one clinical attack compared to 32 the previous year.

The efficiency of the drug in protecting the subjects of this experiment from malaria is further emphasized by the malaria history of the group after treatment was stopped. During the second month after the last drug dosage five members of the group have become infected with *P. falciparum* resulting in clinical attacks of malaria.

EXPERIMENT 4

The effects were tested here of monthly suppressive doses of pyrimethamine in adults and children living under native village conditions in an area unsprayed with residual insecticides. The natives of six small villages were studied. At the outset a house-to-house census was made, the houses numbered and the residents of each house recorded on a separate card by name, sex, and approximate age. In four villages all available persons were treated with pyrimethamine at monthly intervals and a blood film taken from each. In the remaining two villages, situated a half-mile and two miles respectively from the treated villages, placebos were administered and blood films taken; these served as controls. A record of treatment and blood studies was kept for each person in all villages.

The subjects were divided into two groups, a group of children including those under 15 years of age and an adult group which included all the rest. The total

numbers of persons available for study fluctuated from month to month and only those receiving treatment the previous month were included in the treatment group the following month. In the treated villages the numbers of children ranged from 32 to 49, while adults ranged from 72 to 99. In the control villages children examined each month numbered 13 to 24 and adults 32 to 68. Treatment was continued for 18 months and, while no treatment was given on the 19th and subsequent months, members receiving treatment on the 18th month were studied for malaria parasite rates on the 20th and 21st months to determine the rate of the reappearance of malaria following discontinuance of drug administration.

Results. The results are presented in Table 3 and Figure 3. In the treated villages the malaria parasite rate of the children dropped from 46 to 5 per cent by the end of the first month of treatment and, with the exception of one occasion when it rose to 25 per cent, which will be discussed below, it ranged from zero to 9 per cent for the remainder of the treatment period. The mean monthly parasite rate for the group over the 18-month treatment period was 4 per cent. By contrast, the untreated children showed parasite rates ranging from 27 to 73 per cent with a mean monthly parasite rate of 54 per cent. Parasite rates in treated adults fell from 26 to 1 per cent by the end of the first month of treatment and never rose above 3 per cent throughout the treatment period. The mean monthly parasite rate for the group throughout the treatment period was 1 per cent. Untreated adults showed parasite rates ranging from 6 to 35 per cent and the mean monthly rate for the period of observation was 20 per cent.

At the end of the fifth month of treatment the children showed a sudden rise in the parasite rate to 25 per cent. This was unusually high judging by all previous and all subsequent monthly parasite rates and is difficult to explain. It was noted that of the treated children who were found positive for malaria, three were from one family residing in the same house. As they had received drugs when the blood was taken it was considered of interest to re-examine their blood. This was done three days after the drug was administered and by this time the blood proved to be free of malaria parasites. This could indicate that, for some reason, treatment the previous month had been inadvertently missed. Although it is not possible to determine whether or not treatment was missed, this observation is proof that the parasites were not resistant to pyrimethamine.

Treatment was discontinued on the 18th month of the experiment. By the 20th month, or two months after the last treatment, parasite rates in children had risen from 4 to 30 per cent and by the 21st month, or three months after final treatment, had reached a rate of 48 per cent. Adults receiving their last treatment on the 18th month showed parasite rates which rose from 1 to 18 per cent by the 20th month and when examined on the 21st month the parasite rate was 26 per cent.

The rapid reappearance of malaria parasites in the treated subjects of this group as well as in those of *Experiment 3* after the withdrawal of treatment, may be explained either on the basis of reinfection, or recrudescence of latent infection which had been suppressed by the drug. Anneke and Jacobs (1954) who

TABLE 3

Malaria parasite rates in two groups of children and adults living in native villages, one group receiving monthly suppressive doses of pyrimethamine (25 mg.) and the other untreated

	Months																					
	D	-1	-2	-3	-4	-5	-6	-7	-8	-9	-10	-11	-12	-13	-14	-15	-16	-17	-18	-19	-20	-21
Treated Children																						
Number exam.	48	37	39	43	37	32	36	38	38	36	36	38	47	49	48	40	42	45	46		26	25
Number pos.	22	2	0	0	1	8	3	2	2	1	1	0	2	1	1	0	2	0	2		8	12
Per cent pos.	46	5	0	0	3	25	9	5	5	3	3	0	4	2	2	0	4	0	4		30	48
Control Children																						
Number exam.	24	22	17	18	19	24	19	14	12	18	16	13	14	13	24	19	20	18	12		14	18
Number pos.	15	16	11	12	5	13	9	9	4	6	8	7	7	8	13	9	12	9	8		9	10
Per cent pos.	63	73	65	67	27	54	48	64	33	33	50	54	50	61	54	49	60	50	67		64	56
Treated Adults																						
Number exam.	99	97	87	87	86	73	65	76	83	73	72	99	85	82	83	79	75	79	77		60	65
Number pos.	26	1	1	0	0	2	1	0	0	1	2	1	0	2	1	1	0	1	0		11	17
Per cent pos.	26	1	1	0	0	3	2	0	0	1	3	1	0	2	1	1	0	1	0		18	26
Control Adults																						
Number exam.	45	68	41	48	53	43	51	47	45	44	48	37	43	36	50	33	33	39	32		37	48
Number pos.	5	5	5	12	13	17	10	13	9	12	6	7	15	8	11	10	7	4	2		8	13
Per cent pos.	11	7	12	25	25	16	20	28	20	27	13	19	35	22	22	30	21	13	6		22	27

* Drug treatment discontinued

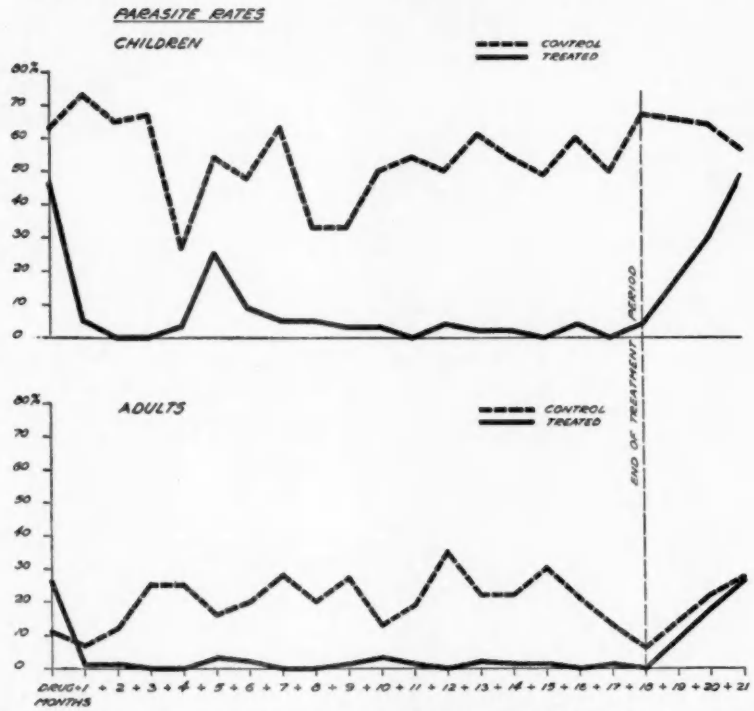

FIG. 3. Malaria parasite rates in two groups of children and adults living in native villages, one group receiving monthly suppressive doses of pyrimethamine and the other untreated.

reported excellent control of malaria in children receiving weekly pyrimethamine in an area where *P. falciparum* was predominant, noted that five weeks after the cessation of treatment there was a rise in the parasite rate from zero to 20.5 per cent. And because they were working in an area of low anophelism and presumably with a low transmission level, they considered the rapid reappearance of the parasites as evidence of recrudescence of latent infections.

Our experiments provide evidence to suggest that the reappearance of parasites after discontinuance of drug treatment was due to reinfection rather than recrudescence. In *Experiment 1*, where the *Anopheles* mosquitos were known to be few and transmission was at a relatively low level, the treated group receiving pyrimethamine was examined two months after the final drug treatment and the parasite rate found to have changed very little, rising from 3 to 5 per cent. On the other hand, in the areas of Experiments 3 and 4, the studies of Fox (1956) of this Institute showed the malaria infection challenge to have been relatively high. He carried out mosquito-population studies and sporozoite-rate determinations in multiple collection sites over a seven-month period during the

course of the experiment, and found infected mosquitos during each of the seven months with sporozoite rates ranging from 1.8 to 5.2 per cent. The treated villages showed infected mosquitos on five of the seven months with sporozoite rates of 0.9 to 4 per cent. A malaria-infection challenge of this order could easily explain the rapid rise in malaria parasite rate following cessation of treatment, on the basis of reinfection.

RESISTANCE OF P. FALCIPARUM TO PYRIMETHAMINE

The apparent frequency with which strains of *P. falciparum* develop resistance to pyrimethamine in East Africa, as indicated by the reports of Jones (1954), Clyde (1954) and Clyde and Shute (1954), is in marked contrast to the situation as it exists in West Africa. In *Experiment 1*, in the group receiving pyrimethamine, we did find one child of three years with asexual parasites before and after drug administration in the fourth month. In the fifth month she was given double the regular dose or 50 mgm. pyrimethamine, and when examined five days later her blood was found to be free of malaria parasites.

The results of our studies do not rule out the possibility that strains of *P. falciparum* resistant to pyrimethamine may be developed or may exist in this area, but we have been unable to isolate such resistant strains. If they exist, resistance to pyrimethamine must be a very much rarer phenomenon here than reports from East Africa would indicate for that area.

SUMMARY AND CONCLUSIONS

Four experiments designed to test the efficiency of monthly suppressive doses of antimalarial drugs for the control of malaria in indigenous West Africans are reported. The results consistently showed that monthly pyrimethamine in 25 mgm. doses to adults and children over three years and 12.5 mgm. doses to children under three years, in the absence of mosquito control measures, was effective in markedly lowering parasite rates and reducing clinical malaria attacks for periods of from one to two years. They further demonstrated that if strains of *P. falciparum* resistant to pyrimethamine occur in this part of West Africa, they are much less prevalent than reports from East Africa would indicate for that area and do not interfere with malaria control under the conditions of the experiments described in this report.

ACKNOWLEDGEMENTS

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STUDIES ON MALARIA IN CHIMPANZEES. IV. *PLASMODIUM OVALE**

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It was stated in a previous communication in this series (Bray, in press) that it was probable that the liver of chimpanzees could support the growth of the tissue stages of the human malaria parasites. Rodhain (1956) and Bray (in press) have shown that this is true of *Plasmodium vivax*. Further, Rodhain (1939) has shown that *P. vivax* can remain in an occult state in an intact chimpanzee for one month. Bray (in press) has shown that this figure can be extended to three and a half months. Bray (in press) and Garnham, Lainson and Gunders (1956) have shown that a splenectomized chimpanzee will allow an infection of *P. vivax* in the blood comparable to that which occurs in non-immune humans.

This communication records similar findings in the susceptibility of chimpanzees to *P. ovale*.

MATERIALS AND METHODS

The strain of *P. ovale* used was indigenous and was isolated locally from a human infection.

The hosts employed were *Pan troglodytes verus* and *Anopheles gambiae*. Chimpanzees were infected by intravenous inoculation of a suspension of sporozoite-infected salivary glands of *A. gambiae* in 20% (v/v) inactivated human serum in Locke's fluid.[1] All chimpanzees were treated with chloroquine 900 mg. base over 3 days and primaquine 105 mg. over 14 days, at least one month prior to experimental use. The mosquitoes used were drawn from an old-established laboratory colony.

The blood phase of *P. ovale* was examined in thick and thin smears stained with Giemsa. Liver pieces were removed from chimpanzees by open laparotomy under ether anesthesia. The pieces were fixed in Carnoy's fluid, sectioned and stained by the Giemsa-colophonium and the Feulgen light-green methods.

RESULTS

The *A. gambiae* fed on the human gametocyte carrier showed a 90% infection with many oocysts, 38.4% of which displayed the typical cross-pigment formation of *P. ovale*. Sporozoites were found in the glands on the 14th day after feeding. On the 16th day after feeding, 9 sets of salivary glands were employed to infect Chimpanzee No. 14 (estimated age 18 months) which had been splenec-

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[1] I am indebted to Dr. R. C. Muirhead-Thomson for assistance in the dissection of mosquitoes.

tomized two months previously. Chimpanzee No. 14 showed a scanty infection or rings 10 days later and rapidly developed a heavy infection with *P. ovale*.

When gametocytes were present 1,000 *A. gambiae* were fed, which became 70% infected, with 1–40 oocysts per gut. Very scanty sporozoites were found in the glands on the 12th day and the glands were heavily infected on the 14th day. On the 15th day after feeding, the glands of 312 of these mosquitoes were inoculated into Chimpanzee No. 16 (intact; estimated age 27 months). A further 8 of these mosquitoes were allowed to feed upon an African child aged 9 years.

Liver pieces were removed from Chimpanzee No. 16 on the 7th, 18th and 39th days after infection, and the animal was splenectomized on the 39th day also.

Chimpanzee No. 16 showed no infection in the blood 215 hours after infection, a few tiny rings 216 hours after infection and a very heavy parasitemia with rings 218 hours after infection. The first generation of erythrocytic forms appeared normal but the second generation was greatly reduced. The third generation was again reduced and the fourth generation was invisible. Thus the blood infection disappeared after 8 days.

The child who received 5 infective bites showed a scanty infection with *P. ovale* on the 14th day after infection.

The liver piece taken from Chimpanzee No. 16, 7 days after infection showed approximately 17,000 pre-erythrocytic schizonts per cu.cm. of liver tissue, the piece taken 18 days after infection showed approximately 600 schizonts per cu. cm., and the piece taken 39 days after infection showed 1 schizont in 100 sections.

The blood infection in the chimpanzee

All stages are typical of *P. ovale* in humans, with the possible exception of the number of merozoites produced. In a total of 50 mature schizonts 2 had 8 merozoites, 11 had 10 merozoites, 20 had 12 merozoites, 12 had 14 merozoites and 5 had 16 merozoites. The nuclei are large and oblong in the schizonts.

The host erythrocyte is frequently oval, sometimes fimbriated and, when parasitized by an older parasite, very pale. The host erythrocyte is stippled and enlarged, though not grossly so, when containing trophozoites, schizonts and gametocytes.

Parasitemia persisted in Chimpanzee No. 14 for 21 days, then became latent and a recrudescence commenced 21 days later, lasting for 2 months; 59 days after the disappearance of the recrudescence a relapse occurred. In Chimpanzee No. 16 no relapse has occurred to date.

The tissue stages of P. ovale *in the chimpanzee*

The 7-day-old pre-erythrocytic schizonts

Four different forms of the schizonts occur regularly. In order of frequency they are first a schizont in which the nuclei do not occur within 1 to 5 μ of the periphery (see Fig. 1). There is a ring of vacuolated cytoplasm around the whole of the periphery of the parasite. Rarely one or two vacuoles are seen in the center portions of the schizont. This form is varied by having sometimes little

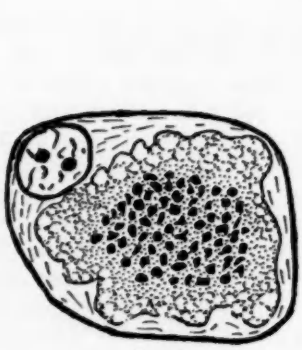

FIG. 1

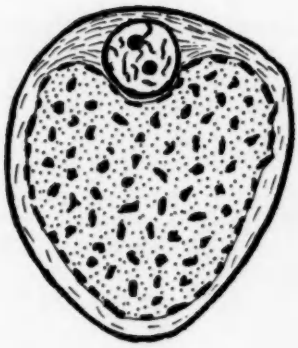

FIG. 2

FIG. 1. A 7-day-old pre-erythrocytic schizont of *P. ovale* showing the central distribution of nuclei and the vacuolation of peripheral cytoplasm. ×1000

FIG. 2. A 7-day-old pre-erythrocytic schizont of *P. ovale* showing the peripheral and tangental distribution of the bar-shaped nuclei. ×1000

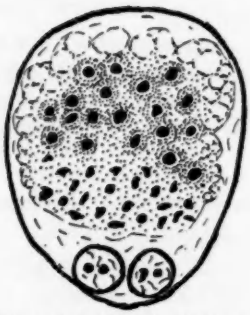

FIG. 3. A 7-day-old pre-erythrocytic schizont of *P. ovale* showing the arrangement of cytoplasm around nuclei giving the psuedo-cytomere appearance. ×1000

or no vacuolation of the peripheral cytoplasm; sometimes the peripheral cytoplasm is discontinuous and the nucleated mass encroaches at points around the periphery.

The second form has a definite peripheral arrangement of a number of bar-shaped nuclei so that the bars lie longitudinally and regularly along the periphery in a line. These clearly demarcate the limits of the schizont with a discontinuous but evenly spaced line of nuclei (see Fig. 2).

The third form is the typical pre-erythrocytic schizont of the *P. cynomolgi* type. It is a mass of unvacuolated cytoplasm with nuclei scattered evenly throughout.

The fourth form may display features of either the first or third forms but the nuclei and cytoplasm are arranged so as to give the impression of cytomere formation (See Fig. 3). The cytoplasm in the neighborhood of a nucleus is frequently arranged about the nucleus in a sphere with nucleus lying in a clearer

space in the middle. These units are not discrete and the intervening spaces are filled with tenuous cytoplasm. The effect is more an impression gained by a relative concentration of cytoplasm around nuclei with less concentrated areas of cytoplasm occurring between the units than a true cytomere formation. The nuclei involved are usually round and faintly Feulgen-positive. One schizont was seen to possess the large almost square nuclei of the *Nycteria* type.

Except where indicated otherwise, above the nuclei are large, bar-shaped or oblong and Feulgen-negative. Many schizonts show a distinct envelope which is not always continuous. In a few it is well marked, continuous and smooth, but more often it is crinkled and apparently discontinuous. The outline of the first, third, and especially the fourth, form shows small convolutions of the outline with clefts apparently just beginning to form. Two major indentations in the outline of the parasite occur. The first occurs to accommodate the host cell nucleus around which the parasite grows in two very blunt lobose arms, sometimes almost encompassing it. The other was seen in two schizonts in a fatty area of liver where the parasite had commenced to grow around a fat globule apparently already present in the host cell.

In general the parasite is spherical to ovoid, fairly regular in shape and measures an average 36.6 μ x 30.3 μ (45–28 μ) x (39–23 μ). Five retarded forms were seen among 200 schizonts and these measure 23 μ x 13 μ, 22 μ x 15 μ, 20 μ x 18 μ, 19 μ x 18 μ and 19 μ x 14 μ.

The host cell of the pre-erythrocytic schizonts

The host cell of the typical 7-day-old schizont is hypertrophied to a greater degree than is necessary to accommodate the parasite alone; thus its nature is easily determined. It is a parenchyma cell greatly enlarged but often containing some cytoplasm unused as yet by the parasite. This residual host cell cytoplasm is pushed to the periphery, making a wall between the parasite and the host cell envelope and piling up particularly in the vicinity of the host cell nucleus, as often occurs with *Isospora*.

The host cell nucleus is also hypertrophied visibly but not greatly. The average diameter of 100 host cell nuclei is 9.6 μ, and, of 500 nuclei of uninfected parenchyma cells is 7.9 μ. The host cell nuclei frequently stain less strongly than the nuclei of uninfected cells. There is no multiplication of nucleoli within the host cell nuclei. Uusually the host cell nucleus, though pushed to the periphery of the cell, is not flattened and the parasite is forced to make accommodation for it.

The schizonts in liver tissue taken 18 days and 39 days after infection

Seven schizonts were found in 177 sections of liver tissue taken 18 days after infection. Their dimensions are as follows:—91 x 50 μ, 69 x 44 μ, 62 x 45 μ, 61 x 44 μ, 52 x 36 μ, 51 x 37 μ and 40 x 31 μ.

The first schizont is very large and has a regular outline. The nuclei are closely packed in the schizont and are of the *Nycteria* type, that is, large and square. There is no peripheral chromatin or vacuolation of the peripheral cytoplasm.

All the remainder show the zone of vacuolated cytoplasm at the periphery to a greater or lesser degree. Two show a pronounced tendency to form islands of cytoplasm, each containing a nucleus. The cytoplasm becomes a network with the nuclei lying in the spaces. All schizonts (except the first above) display a peripheral distribution of chromatin but not in the form of nuclear bars. The chromatin is deposited as very large masses (up to 7 μ across) irregularly on the envelope of the schizont. The effect is similar to that stage of the sporont of the Adeleida where the large nuclei have migrated to the periphery prior to sporoblast formation. However, these masses are referred to as chromatin rather than nuclei, as they are a light scarlet red when stained with Giemsa. They never show the dark dense reds and purples of the frank nuclei and do not appear, therefore, to be generative in nature. (See Fig. 4.)

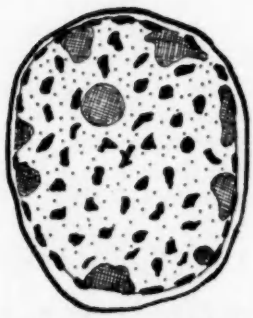

FIG. 4. A second generation exo-erythrocytic schizont of *P. ovale* from liver tissue taken 18 days after infection. Note the large masses of peripheral chromatin. ×800

In 3 of the schizonts one large vacuole is present which is filled with a substance apparently colloidal and which stains pink to deep red depending on the depth of material.

In general, the envelope is plain to see and there is little or no convolution of outline. The nuclei resemble those in the 7-day-old forms.

The schizont found in the liver tissue taken 39 days after infection measures 33 μ x 31 μ and is typical of the third form of the 7-day-old schizonts described above. The nuclei are bar-shaped and spherical and some stain a deep purple with Giemsa.

DISCUSSION

P. ovale behaved in a similar fashion to *P. vivax* in the chimpanzee. The liver of the chimpanzee provides an excellent medium for the growth of the exo-erythrocytic forms of *P. ovale*. Similarly, the splenectomized chimpanzee is an excellent experimental host for erythrocytic schizogony and gametogony of *P. ovale*.

A. gambiae is established as an efficient vector of *P. ovale* and is undoubtedly one of the main vectors of *P. ovale* in West Africa.

It would appear that splenectomy of the chimpanzee has little effect upon the number of pre-erythrocytic schizonts resulting from any given number of sporozoites. The number of sporozoites used in the present experiment was of the same order as that used previously (Bray, in press) for the infection of a splenectomized chimpanzee with *P. vivax*. The numbers of pre-erythrocytic schizonts found on both occasions are roughly equal. Furthermore, by the same analogy, splenectomy seems to have no effect upon the numbers of second generation exo-erythrocytic forms.

On the other hand, splenectomy of the chimpanzee has a considerable effect upon the growth of the erythrocytic forms of *P. ovale*. The intact chimpanzee was able to reduce parasitemia to below visible levels in 8 days and at no time was an increase in parasite numbers observed. Despite subsequent splenectomy no relapse has occurred to date. In contrast, the splenectomized chimpanzee allowed an increasing parasitemia for 10 days with all erythrocytic forms and the parasites were visible for 21 days.

P. ovale retained its specific characteristics throughout the infections in chimpanzees, showing oval and stippled host erythrocytes and compact forms with large nuclei in the erythrocytic phase, the cross pigment formation in 38.4% of the young oocysts in the arthropod phase, and the hypertrophied host cell nuclei and large parasite nuclei in the hepatic phase. The record of 12 days for the development of sporogony, although the lowest recorded in the literature, nevertheless compares with the figures of 9 days recorded here for *P. vivax* and *P. falciparum* in *A. gambiae*. The duration of the pre-erythrocytic generation (9 days exactly) is in accord with that found by Garnham *et al.* (1955). After two passages directly through the chimpanzees and *A. gambiae* the strain was able to infect man.

The pre-erythrocytic schizonts of *P. ovale* displayed three characteristics not noted previously in infections with *P. vivax*, *P. cynomolgi*, *P. inui* or *P. falciparum*. Firstly, the presence of a definite limiting membrane or periplast in many of the schizonts, as noted by Garnham et al. (1955) and in the present study. Secondly, the peripheral arrangement of the nuclear bars in some schionts which is tangential, as distinct to the radial "palisade" arrangement seen with *P. inui* (Garnham, 1951). Thirdly, the minor but visibly distinct hypertrophy of the host cell nucleus and its resistance to the pressure of the schizont, also noted by Garnham et al. (1955).

The size of the 7-day-old pre-erythrocytic schizont of *P. ovale* at 36.6 μ x 30.3 μ is perhaps a little smaller than the same aged form of *P. vivax* (42 μ in the longest diameter, Shortt and Garnham, 1948) but much of a size with *P. cynomolgi* of the same age (36 μ av. diameter, Bray, 1957). It is distinctly larger than *P. inui* of the same age (7–8 μ diameter, Garnham, 1951).

The size of the nuclei said to be larger in the "fifth-day schizonts" of Garnham et al. (1955) are, at the 7-day-old stage, visibly larger than those of similar forms of *P. vivax*. The reason for taking liver on the 7th day after infection was to attempt to fix the true position of the "fifth-day schizonts" of Garnham et al. (1955). This age designation was said to be provisional by the authors and

it can now be said to be almost certainly wrong. Of the "fifth-day schizonts" of Garnham et al. (1955) the smallest measured 28 μ in length, 5 ranged from 40–45 μ and the 2 largest measured 60 μ. The first may be a true 5-day-old form or, less likely, a retarded form, the remainder are probably older than 7 days and it may be that the 5 ranging from 40–45 μ are 8-day-old forms and the 2 largest may be 8 or 9-day-old forms. Another reason for believing that these last forms are older than reported is that their nuclei were said to be strongly Feulgen-positive which is not true of the 7-day-old forms.

At 7 days of age the pre-erythrocytic schizonts of *P. ovale* have not begun to show the excessive lobulation shown by the later stages but the pseudo-cytomere or "coccidia-like" arrangement of the cytoplasm around individual nuclei is marked in some 7-day-old forms. The tangential arrangement of the nuclei is also more marked than the "bead-like rows on the limiting membrane" observed by Garnham et al. (1955) in the 9th day forms. Of the schizonts seen in the liver tissue taken 18 days after infection, the largest one might well be a retarded first generation form. The remainder which, by analogy, would appear to be 7 to 8 days old, are thought to be second generation forms for the same reasons as were advanced in the case of *P. vivax* in the chimpanzee liver 15 days after infection (Bray, in press). The predominant feature which these schizonts display in common is the large chromatin masses to be found at the periphery of the parasite which have not been seen in the first generation forms. The purpose of this arrangement remains unexplained at present.

The demonstration of a schizont in the liver 39 days after infection demonstrates conclusively the persistence of the tissue phase of *P. ovale*. The stain reaction of the nuclei point to a state of active division and growth in the schizont and there is no evidence that it is a resting stage.

Liver was taken at the 39th day to determine if the natural resistance of the intact chimpanzee to the blood forms of *P. ovale* affected the exo-erythrocytic cycle of the parasite in the same manner as the acquired immunity of *Macaca mulatta* to *P. cynomolgi* affected the exo-erythrocytic cycle of that parasite. Garnham and Bray (in press) have shown that an acquired immunity, while having no effect upon the first generation of the tissue phase, causes a considerable increase in the numbers of the later generations.

This was not the case in the present study with Chimpanzee No. 16 where the natural resistance to the blood phase of *P. ovale* did not cause any large increase in the numbers of the later generations of the tissue phase.

SUMMARY

The chimpanzee displays a natural resistance to the blood forms of *Plasmodium ovale* but allows multiplication and growth of this phase when splenectomized.

The tissue phase of *P. ovale* is able to grow and reproduce normally in chimpanzee liver and persists there for at least 39 days. The natural resistance of the chimpanzee to the blood phase is without effect upon the tissue phase of *P. ovale*.

P. ovale is unchanged on passage through chimpanzees and is able to infect man following two chimpanzee-mosquito passages.

Anopheles gambiae is established as an efficient vector of *P. ovale*.

Schizonts of *P. ovale* in liver pieces of a chimpanzee taken 7, 18 and 39 days after infection with sporozoites are described and discussed.

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THE EFFECT OF PENICILLIN G ON TWO STRAINS OF SMALL RACE *ENTAMOEBA HISTOLYTICA* IN CULTURE[1]

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That there is great variation in the size of the amebas classified as *Entamoeba histolytica* has long been recognized (Quinke & Roos, 1893; Vierick, 1907; Walker, 1911; Ujihara, 1914; Dobell & Jepps, 1918). Most observers appear to agree that there are at least two races of *E. histolytica*, a small race with cyst sizes averaging 10 microns or less and a large race with cyst sizes averaging over 10 microns (Walker, 1911; Sapero, *et al.*, 1942). The differentiation into races has been based completely on size, with 10 microns as an arbitrary dividing line. Little information is available on comparative, cultural or other fundamental characteristics of the various sizes of these amebas.

Strains of large race *E. histolytica* have been grown in culture with relative ease since the original successful experiments of Boeck and Drbohlav (1925) and have been studied extensively by numerous investigators (Dobell and Laidlaw, 1926; Cleveland and Sanders, 1930; Rees, 1956; Jacobs, 1947; Shaffer and Frye, 1948; Shaffer *et al.*, 1948; Shaffer 1952; Meleney and Frye, 1936; Frye and Meleney, 1938; etc.).

There are few reports on studies on small race strains. Drbohlav (1925) reported the successful cultivation of a strain of ameba which he called *Tetragena minuta*. He maintained the culture for over a month, but it was lost, apparently due to overgrowth of *Blastocystis hominis*. Frye and Meleney (1938) isolated a small race (Denton) and maintained it for several years, but it later changed to a large race (Meleney and Zuckerman, 1948). Sapero *et al.* (1942) were unsuccessful in 100 attempts at culturing small race *E. histolytica* using liver infusion media, although they were successful in 80 per cent of their attempts with the large race. The authors made 50 attempts to isolate small race amebas using Locke egg slant, Cleveland-Collier liver slant and Balamuth media. Only one successful isolation was made and a strain (Conrad) established in liver slant medium. This culture has been maintained for approximately 2 years. McQuay (1956) has described a medium containing numerous chemicals in which he has isolated several strains of small race *E. histolytica* from feces with considerable ease. This has made small race cultures readily available for comparative studies.

The present report describes some interesting observations which apparently indicate differences between two strains of small race and two strains of large race *E. histolytica* in their response to penicillin G (Penicillin G, Potassium, Squibb).

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EXPERIMENTAL

These observations are an outgrowth of attempts to transfer strains of small race *E. histolytica* from mixed bacterial flora in diphasic (Locke egg slant or liver slant) medium to the Shaffer-Frye (S-F) medium (Shaffer *et al.*, 1948b). Several different strains were tested and none were found capable of establishment in S-F medium. Numerous variations of technique to accomplish this were tried, but none succeeded.

Almost invariably, when an inoculum of a small-race strain was transferred from diphasic medium into the S-F medium, there was a marked increase in cells in the S-F cultures during the first 24 hrs. of incubation at 37°C., giving the impression that active growth and multiplication had occurred. However, all attempts at further transfer in S-F medium resulted in failure. On more careful examination of the cells seen in the initial S-F cultures, it was found that they were not typical amebas, being nonmotile with an amorphous cytoplasm surrounded by a band of nuclear-staining material. These structures bore a striking resemblance to *Blastocystis hominis*.

In one set of experiments, still attempting to establish small-race *E. histolytica* in S-F medium, Aureomycin HCl in concentrations varying from 0.01 to 0.0003 mg./ml. (Fuller and Faust, 1949; Shaffer and Beigeliesen, 1951) was substituted for penicillin G. Under these circumstances, the marked increase in cells did not occur, but the cells that were present were typical amebas. No evidence of propagation was seen, however, since serial transplants were unsuccessful.

It was felt that the above observation with Aureomycin HCl might indicate that penicillin G was responsible for the peculiar result obtained with S-F medium. Accordingly, penicillin G in concentrations similar to that used in S-F medium (250 to 300 u/ml.) was added to cultures of the Conrad[2] and Griffith[3] strains of small race *E. histolytica* in mixed bacterial flora. With considerable regularity, there appeared *Blastocystis*-like structures in these cultures when examined at the end of 24 hours' incubation at 37°C. It was decided at this point to limit further studies to the Conrad and Griffith strains since they seemed to be typical of this group of small race amebas.

In attempting to explain the origin of these *Blastocystis*-like structures, three possibilities were considered: (1) they might actually be *Blastocystis hominis*, present in the Conrad and Griffith cultures in small numbers and stimulated to active propagation by the penicillin G; (2) they might be derived from one or more members of the bacterial flora, developing under the influence of the penicillin G; or (3) they might be degenerate *E. histolytica* trophozoites, resulting again from the influence of the penicillin G.

The first possibility was essentially ruled out in two ways: first, extensive

[2] The Conrad strain was isolated in the Parasitology laboratory of The Hektoen Institute, Cook County Hospital into diphasic medium from the feces of a patient with chronic symptomatic amebiasis. Only small race cysts were seen in the specimen.

[3] The Griffith strain was isolated by Dr. McQuay at The Mt. Sinai Hospital, Chicago in his special medium, from the stool of a patient with chronic symptomatic amebiasis.

studies of routine transplants of both strains of small race *E. histolytica* without penicillin G were made. Careful microscopic study of stained and unstained preparations failed to reveal any structures resembling *Blastocystis hominis*. The stained preparations were made with a modified Mallory's phosphotungstic acid stain (Felsenfeld and Young, 1945). Second, inoculation of the Conrad and Griffith strains into Barret's (1921) medium, in which *B. hominis* appears to grow readily, failed to produce cultures of *B. hominis*.

The second possibility was ruled out by isolating the bacterial flora free of amebas and studying the flora microscopically with appropriate stains through 12 successive transplants with and without penicillin G. Penicillin G concentrations were 260 u/ml. in each case. At no time were any *Blastocystis*-like structures seen.

Thus, the third possibility remained. Slides were prepared from cultures of the Conrad and Griffith strains of *E. histolytica* grown in the presence of varying concentrations of penicillin G in the diphasic medium. These slides were stained with the modified Mallory's phosphotungstic acid stain and studied carefully. In many of these preparations, depending on the concentration of penicillin G employed as will be shown later, there appeared cells which varied in appearance from the typical *E. histolytica* trophozoite to the *Blastocystis*-like body. Thus, it appeared likely that these *Blastocystis*-like structures were, indeed, degenerate amebic trophozoites. Indication that these structures were not *B. hominis* was obtained when, on inoculation into Barret's medium, no growth occurred.

The first evidence of degeneration in the amebic trophozoite was the appearance of vacuoles in the cytoplasm. There might be one or several. These vacuoles gradually enlarged and filled more and more of the cell until the nucleus was pressed against the cell wall. The final stage appeared to be fragmentation of the nucleus, resulting in a structure with a clear, amorphous center surrounded by a ring of presumably nuclear material. This was the stage that most closely resembled *B. hominis*.

In Figure 1 are photomicrographs of some of the organisms discussed above. Figure 1a is a typical small race *E. histolytica* trophozoite from a culture; 1b, c and d are some stages of degeneration of small race trophozoites in the presence of penicillin G; 1e is a *Blastocystis hominis* in a fecal smear; and 1f and g are *B. hominis* from culture in Barret's medium.

Since it now appeared that these structures were degenerate amebic trophozoites and that penicillin G was in some way involved with their production, it was of interest to examine the effects of varying concentrations of penicillin G on cultures of the small-race Conrad and Griffith strains and compare the results with similar observations on two typical large-race strains of *E. histolytica* in mixed bacterial flora. The two strains of large race *E. histolytica* used in these experiments were K-9[4] and 200[5].

[4] The K-9 strain was obtained from Dr. M. M. Brooke of the Communicable Disease Center, Public Health Service. It was brought from Korea in 1951 by Dr. W. W. Frye.

[5] The 200 strain was obtained from Mr. Max McCowen of the Lilly Research Laboratories. It was obtained originally from Dr. C. W. Rees of the National Institutes of Health.

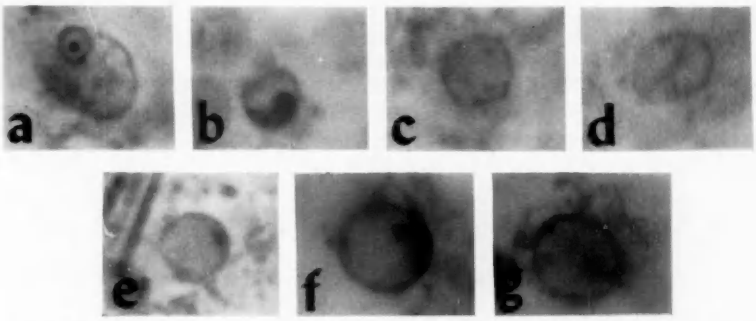

FIG. 1. (*a*) Typical small race E. histolytica trophozite in culture; (b) Partially degenerated small race trophozoite after exposure to penicillin G; (c) and (d) Degenerated small race *E. histolytica* trophozoites with apparently fragmented nucleus resulting from exposure to penicillin G; (e) *Blastocystis hominis* in stool specimen; (f) and (g) *B. hominis* from culture in Barret's medium. Magnification about 2000×. Stained with modified Mallory's Phosphotungstic Acid stain (Felsenfeld & Young, 1945).

Two diphasic media were used in these experiments. The large-race strains (K-9 and 200) and the small-race Griffith strain were grown on egg slants with Locke's solution overlay, and the small-race Conrad strain was grown on liver slants with an overlay of human serum-Locke's solution, 1:8. A small, standard amount of rice powder was used routinely. There are obvious objections to the use of different media in comparative experiments, but no other course was available since the Griffith strain did not grow well in liver-slant medium and Conrad did not grow well in egg-slant medium. Preliminary evidence indicated that the most important thing was to have a medium in which the organisms propagated well.

The experiments were set up as follows: a series of 9 tubes of the appropriate medium were used for each strain of *E. histolytica*. The overlay was added to each tube in a volume of 3.5 ml. per tube just before inoculation with amebas. Varying concentrations of penicillin G were added to 8 of the tubes in a volume of 0.2 ml. in amounts calculated to provide final concentrations as shown in Table 1. The 9th tube was a control with no penicillin G, but with 0.2 ml. of additional overlay to make the volume constant. Inoculation of the cultures was accomplished by transferring 0.1 ml. from the slant-fluid juncture of 48 hr, 4+ cultures of the respective strains to each of the experimental tubes. Care was used to provide as nearly uniform inocula as possible but no counts were done to determine the exact numbers transferred. After inoculation, the tubes were incubated at 37°C. for 48 hrs. At the end of this period, samples were removed to slides—one slide for direct microscopic examination to estimate comparative growth and reproduction, and two slides to be fixed, stained and examined for the presence or absence of *Blastocystis*-like bodies.

In Table 1 are shown the average of results obtained in 3 separate experiments on the 4 strains of *E. histolytica*. It will be seen that, under these conditions, propagation of the Conrad strain (small race) was inhibited by all concentrations

TABLE 1

Effect of penicillin G on strains of small and large-race Entamoeba histolytica *in diphasic medium*

Entamoeba histolytica strain	Penicillin G concentration (units/cc.)	Amebas	Results (48 hrs) Blastocystis-like bodies
Conrad (small)	1316	1+*	4+
	658	1+	2+
	329	—	1+
	263	1+	1+
	219	±	1+
	164	1+	1+
	132	1+	1+
	82	1+	—
	—	4+	—
Griffith (small)	1316	1+	1+
	658	±	1+
	329	1+	—
	263	1+	—
	219	2+	—
	164	2+	—
	132	4+	—
	82	4+	—
	—	4+	—
K9 (large)	1316	4+	—
	658	4+	—
	329	4+	—
	263	4+	—
	219	4+	—
	164	4+	—
	132	4+	—
	82	4+	—
	—	4+	—
200 (large)	1316	2+	—
	658	2+	—
	329	2+	—
	263	3+	—
	219	3+	—
	164	1+	—
	132	1+	—
	82	1+	—
	—	3+	—

* Readings: 1+ = 1 to 10 amebas/low power field; 2+ = 11 to 25 amebas/low power field; 3+ = 26 to 50 amebas/low power fields; 4+ = 50+ amebas/low power field

of Penicillin G, although even the highest concentration did not result in negative readings. Concentrations of penicillin G of 132 u/ml. and higher resulted in the production of *Blastocystis*-like bodies, with increased numbers in the presence of 658 and 1316 u/ml. With the Griffith strain (small race), inhibition of propagation occurred with 164 units of penicillin G/ml., but *Blastocystis*-like bodies were

present only in the tubes with 658 and 1316 u/ml. Strain K-9 (large race) was unaffected by any of the concentrations of penicillin G and strain 200 (large race), although its propagation was irregular during the time these experiments were being done, was apparently not affected by the penicillin G.

Thus, under the conditions of these experiments there were marked differences in the effect of penicillin G on the small and large-race cultures and there was some difference in response between the two-small-race cultures. How much of this latter difference was the result of the use of different media cannot be determined at the present time.

It is impossible to state definitely whether or not the effects of the penicillin G were exerted directly on the amebas or were secondary to some influence on the bacterial flora of the two small-race strains of *E. histolytica*. Certain observations favor the view that the effect was directly on the amebas, although they are certainly not conclusive.

(1) The various individual members of the bacterial flora of the two small-race strains were sensitive to 30 to 60 units of penicillin G per ml. when tested individually, but when tested as a flora, there was little, if any evidence of marked effects even with concentrations of penicillin G as high as those shown in Table 1. No ready explanation of this observation can be offered at present. Although shifts in the relative numbers of the various members of the flora may have occurred, none was removed from the flora by the penicillin G.

(2) Experiments on the bacterial flora of the large-race strains indicated a situation quite similar to that in the small-race strains, regarding the effect of penicillin G on the flora.

(3) The changes noted in the amebic trophozoites when attempts were made to transfer the small race strains into S-F medium did not occur when Aureomycin was substituted for penicillin G.

In a number of experiments, transfers were made from the various tubes in each series to similar tubes containing the same concentrations of penicillin G. In all cases where 2+ to 4+ amebas were present, transfers were accomplished with ease, but successful transfers were irregularly accomplished from tubes containing 1+ or less amebas. In almost all cases, however, it was found that transfer to medium without the antibiotic resulted in normal propagation of the amebas if any were present in the original tube.

DISCUSSION

The observations described above are of interest for at least two reasons. First, this appears to represent a difference between these particular strains of small and large-race *E. histolytica*. It will take further study to determine whether or not this difference is consistent between the races. Further studies of these strains using other antibiotics and certain of the well-known ambecides are now in progress and it is planned to study the effect of penicillin G on other small race strains.

The second point of interest is the peculiar form of degeneration which results in structures resembling *B. hominis*. This observation stimulated considerable

interest in *B. hominis*. A survey of the literature revealed that a great deal of investigative work has been done in the past on this organism, although the recent literature contains but few references. Some workers reported cases in human beings in which they felt that *B. hominis* showed evidence of pathogenic properties (Garcia, 1929; Calderin, 1937). It would be interesting to know whether or not these authors were dealing with degenerating small race *E. histolytica*, if, indeed, the kind of degeneration observed here can occur in the intestinal tract. It is to be hoped that interest in *B. hominis* will be renewed and its nature and relationships to other intestinal organisms elucidated.

It is essential that much further study be directed towards comparisons between small and large-race *E. histolytica*. It seems likely that there are cultural differences since, as pointed out previously, the small race does not grow well on first isolation in media in which the large race can be quite successfully isolated. Failure of the small-race strains to propagate in the S-F medium also would seem to indicate cultural differences. The similarities and differences between the races need more careful evaluation and tabulation with the hope that better understanding of the disease conditions resulting from infection with these agents will be forthcoming. Exact results are difficult to obtain with the cultural methods now available, but much can nevertheless be accomplished.

SUMMARY

A peculiar type of degeneration has been observed in cultures of small race *E. histolytica* in the presence of penicillin G, yielding structures resembling *B. hominis*. Penicillin G was inhibitory to the propagation of cultures of two strains of small race *E. histolytica* in mixed bacterial flora in concentrations which had no effect on similar cultures of two large race strains.

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REACTIONS TO CHLOROQUINE OBSERVED DURING THE TREATMENT OF VARIOUS DERMATOLOGIC DISORDERS

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Many workers in tropical medicine are not aware of the popularity of chloroquine[1] in modern dermatologic therapy. After the initial success of this compound in the treatment of discoid lupus erythematosus (Goldman *et al.*, 1953), we, in common with many dermatologists, have used chloroquine in a variety of completely unrelated dermatoses. At present, chloroquine is used routinely in the treatment programs of so-called subacute lupus erythematosus, of discoid lupus erythematosus, rosacea, localized scleroderma, solar dermatitis, lymphocytosis faciei, often in acne, and occasionally in sarcoidosis. The daily dose varies from 250–750 mg. Usually, the treatment programs are prolonged in terms of weeks or months. In some cases of disseminated discoid lupus eythematosus, patients have been taking 250–500 mg. of chloroquine daily for three years with only occasional and brief rest periods. It is pertinent, then, to review our experiences of reactions to this compound during the past four years.

When compared with other chemotherapeutic agents used for chronic and resistant dermatoses, chloroquine has been found to be a compound of relatively low toxicity. Actually, millions of tablets of chloroquine have been used in the past 10 years in malaria control programs (Meto Carcedo, 1956). It should not be forgotten, however, that many of the therapy programs in malaria are of brief duration, quite unlike those in dermatologic diseases. The problems of the dermatologist are therefore mainly those of chronic toxicity.

In spite of the widespread use of chloroquine in dermatology, too little is known of its actual mechanism for the relief of various cutaneous reactions. It interferes with the metabolic activity of hexokinase and the yellow enzymes (Rothman, 1955, personal communication), and most of the studies in dermatology concern protection of the skin from light. Preliminary skin assay studies carried out with Cole, Hughes and Schmidt (personal communication, 1953) have shown persistency of chloroquine in tissues after oral doses, with concentration in the skin greatly exceeding that in the plasma. In one patient with subacute lupus erythematosus, the concentration in a lesion area was not greater than in her normal skin.

In our experience, the reactions to chloroquine may be classified in order of severity as:

1) *minor or uncomfortable reactions*—anorexia, weight loss, nervousness, nausea and emesis, and difficulty in visual accommodation;
2) *moderately severe reactions*—transient maculo-papular reactions and achromotrichia;

[1] The Aralen and Plaquenil used in this study were supplied through the courtesy of the Medical Research Department of Winthrop Laboratories, New York.

3) *severe reactions* (uncommon)—major drug reactions (lichenoid types, exfoliative dermatitis), liver damage, and hematopoietic reactions—especially leucopenia.

In general, our experiences parallel those observed in malaria programs (Findlay, 1951) and chronic toxicity studies reported in the literature (Alving *et al.*, 1948; Fitzhugh, 1948). Most of our reactions fall into Group 1. Many may disappear with mere reduction in dosage without interruption of medication. One uncommon reaction in this group developed in a patient with discoid lupus erythematosus following the use of a radium plaque on a lesion of the nose. After only two doses of chloroquine (500 mg.), the patient complained of weakness, fever, nausea, prostration and some redness of the lesions. This reaction disappeared within 24 hours after the medication was stopped, and the lesions became more pale. The medication was not resumed, but later, hydroxychloroquine (Plaquenil), 400 mgs. twice daily, was well tolerated.

The most disturbing symptoms in Group 1 were the eye reactions which however always cleared up completely. In two patients with so-called subacute lupus erythematosus, chloroquine was at first suspected of producing severe fundal changes, but this could not be proved. In both of these patients severe constriction of the visual fields developed. One patient died of acute disseminated lupus erythematosus. The eyes were removed and sectioned but no definite cause for this visual field disturbance could be found. Visual field examinations are being done on patients with long maintenance dosage routines.

Early in our experience, we used hydroxychloroquine to resume therapy in patients unable to tolerate chloroquine. Hydroxychloroquine was more easily tolerated than chloroquine, although larger doses were required to maintain the same degree of clinical response. We have had only two patients who could not tolerate hydroxychloroquine but could take chloroquine.

Next in frequency to the reactions in Group 1 were drug eruptions. The maculopapular reactions were more common than the more severe lichenoid and exfoliative types. All varieties of response to the two drugs were found in this group of drug hypersensitivity: recurrence or failure to recur with renewal of chloroquine; prompt recurrence or failure to recur with hydroxychloroquine.

As a rule, the severe exfoliative reactions were observed when intensive therapy was maintained in the presence of a persistent and active maculo-papular reaction. Pruritus did not always herald the development of a visible drug eruption. We observed no methemoglobinemia during or after any of the drug eruptions. One of the most puzzling cases was that of a psoriatic who developed a maculopapular eruption confined to exposed areas of the skin after only two days of therapy, 500 mg. daily. Therapy was discontinued at once but the eruption became vesicular. It subsided somewhat under meticorten therapy, 15–20 mg. daily, but developed into psoriatic lesions. Limitation of the eruption to exposed areas of the body without improvement of the psoriasis makes this different from the reaction to chloroquine in psoriasis reported by Ziprkowsky (quoted by Witten and Sulzberger, 1956). We had no generalized lichenoid dermatitis in our series. No eczematous contact dermatitis was observed in cases in which

FIG. 1 Band type of achromotrichia associated with chloroquine therapy in a young child with linear scleroderma. Hair color returned gradually while patient was continued on chloroquine.

chloroquine was applied as topical medications for discoid lesions of lupus erythematosus.

Bleaching of the hair, partial or complete, occurred in only two cases of our series—one in a child with linear scleroderma; the other in an adult with lymphocytosis faciei. The color returned to the child's hair even though chloroquine was continued. It is possible that chloroquine may be an antagonist to the antiachromotrichial vitamins.

In cases of lupus erythematosus with major visceral reactions of Group 3, it was always difficult for us to decide whether these were due to the disease or to the chloroquine. Welsh (personal communication, 1954) observed one case of leucopenia in a patient with discoid lupus erythematosus following chloroquine therapy. We have used chloroquine and hydroxychloroquine in acute disseminated lupus erythematosus in an attempt, sometimes temporarily successful, to cause the L.E. factor to become negative and sometimes to wean the patient from corticosteroids. These latter attempts were uniformly unsuccessful. It is in this group and in the subacute group that we have felt most concern regarding major visceral reactions. In ambulatory patients with so-called subacute lupus erythematosus, we prefer to start the patient on chloroquine rather than on

corticosteroids and to continue with it as long as we can. For the less severe reactions of Group 1 or 2 in this phase, we switch promptly to hydroxychloroquine and try to maintain a dosage of 400 mg., equivalent to 250 mg. of chloroquine. In animals, the oral dose-toxicity of hydroxychloroquine is said to be only ⅕ that of chloroquine (Meto Carcedo, 1956). As yet, no leucopenia or liver damage has been reported with hydroxychloroquine.

SUMMARY

Chloroquine as used commonly in dermatologic practice has relatively low toxicity in spite of prolonged course of therapy. The most frequent reactions are minor disturbances such as anorexia, nausea, weight loss, nervousness and difficulty in accommodation. Less frequent reactions are maculo-papular drug eruptions and achromotrichia. The rarer complications are major drug eruptions and visceral reactions. Attention to minor reactions will often prevent more serious reactions later. Hydroxychloroquine (Plaquenil) is usually, but not always, more easily tolerated than chloroquine.

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CHEMOTHERAPY OF *TRYPANOSOMA GAMBIENSE* AND *TRYPANOSOMA RHODESIENSE* INFECTIONS IN GUINEA PIGS WITH NITROFURAZONE

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The curative effect of nitrofurazone (Furacin) on acute *Trypanosoma gambiense* and *Trypanosoma rhodesiense* infections in mice was first demonstrated by Packchanian (1955). The object of the present communication is to describe its curative effect in chronic *T. gambiense* and subacute *T. rhodesiense* infections in guinea pigs.

MATERIAL AND METHODS

The drug. Nitrofurazone, 5-nitro-2-furaldehyde semicarbazone, (Furacin),[1] is a yellowish powder slightly soluble in water and very stable. The LD/50 orally for rats is about 450 mg./kg. Normal guinea pigs tolerated 20 mg./kg. of the suspension daily intraperitoneally for 17 days without any local pathology.

The strains of Trypanosoma gambiense. Eleven strains of *T. gambiense* were used in this study and have been designated as E, K, L, M, Q, R, T, U, X, Y, and Z. All of these strains were isolated during the last five years in the region of Leopoldville, Belgian Congo, from different human patients and have been maintained in guinea pigs by syringe-passage; these strains had undergone from 4 to 88 serial passages in guinea pigs prior to their use in chemotherapy with nitrofurazone.

Each of these strains produced a chronic infection in guinea pigs. After the incubation period, the trypanosomes were readily demonstrable in scanty numbers in the circulating blood of guinea pigs nearly every day. The average survival of infected, untreated guinea pigs with the above strains was about 150 days.

The strains of Trypanosoma rhodesiense. The seven trains of *T. rhodesiense* used in this study have been designated as A, B, C, D, F, G, and H. All of the above strains were isolated from different human patients who had contracted the infection in the first outbreak of *T. rhodesiense* sleeping sickness in Ruanda-Urundi, Belgian Congo, and were maintained in guinea pigs by serial syringe-passages.

T. rhodesiense infection in guinea pigs reared at Leopoldville nearly always runs a subacute course; the mean survival time of infected, untreated guinea pigs with the above strains was about 50 days.

Experimental animals and methods of treatment. Thirty-two healthy guinea pigs were inoculated with eleven strains of *T. gambiense* (one to seven guinea pigs with each strain), and 30 guinea pigs with seven strains of *T. rhodesiense* (one to five guinea pigs with each strain). The weight of these animals at the time of

[1] Received through the courtesy of Eaton Laboratories, Norwich, New York.

TABLE 1

Chemotherapy of chronic Trypanosoma gambiense *infection in guinea pigs with nitrofurazone (Furacin)*

Infection of G. Pig with *T. gambiense* Before Chemotherapy					Chemotherapy with Furacin, Single Dose, mg./kg., I.M.	Results Following Chemotherapy		Conclusion
T. gambiense		Guinea pigs				1st relapse of trypanosomes in blood (No. days)	Neg. microscopic findings for trypanosome (No. days)	
Strain No.	No. syringe-passages in g. pigs	No.	No. days infected	Tryps. in bl.				
E	4	1	31	+	50	35	—	Suppressive
L	51	2	37	+	50	40	—	Suppressive
M	82	3	31	+	50	47	—	Suppressive
X	29	4	34	+	50	37	—	Suppressive
Z	25	5	39	+	50	—	365	Curative
E	6	6	32	+	75	31	—	Suppressive
L	67	7	33	+	75	—	365	Curative
M	78	8	33	+	75	328	—	Suppressive
X	28	9	32	+	75	—	301	Curative
Z	24	10	39	+	75	—	365	Curative
E	7	11	31	+	100	—	254	Curative
K	28	12	39	+	100	—	365	Curative
L	40	13	28	+	100	30	—	Suppressive
L	41	14	19	+	100	—	365	Curative
L	60	15	33	+	100	—	365	Curative
M	86	16	31	+	100	28	—	Suppressive
Q	19	17	57	+	100	29	—	Suppressive
R	34	18	206	+	100	106	—	Suppressive
T	42	19	33	+	100	20	—	Suppressive
U	24	20	216	+	100	21	—	Suppressive
X	19	21	130	+	100	11	—	Suppressive
Y	21	22	105	+	100	19	—	Suppressive
Z	23	23	29	+	100	—	168	Curative
E	8	24	32	+	125	—	365	Curative
L	61	25	32	+	125	—	365	Curative
X	26	26	31	+	125	—	365	Curative
Z	22	27	28	+	125	—	365	Curative
E	5	28	31	+	150	—	365	Curative
L	63	29	33	+	150	31	—	Suppressive
M	88	30	33	+	150	35	—	Suppressive
X	25	31	33	+	150	—	365	Curative
Z	26	32	33	+	150	—	335	Curative

chemotherapy ranged from 147 to 490 grams; most of the animals weighed about 200 to 300 grams each. The guinea pigs infected with *T. gambiense* or *T. rhodesiense* were usually treated for 30 days following inoculation. The longest periods of infection in guinea pigs from the day of inoculation to the day of therapy were 105, 206, and 264 days with *T. gambiense* and 40, 55, and 63 days with *T. rhodesiense*. Trypanosomes were readily demonstrable microscopically in the peripheral blood of all the guinea pigs on the day of chemotherapy. Each of the guinea pigs was treated with one dose of nitrofurazone suspension intramuscularly, the dose varying from 50 to 150 mg./kg. body weight (Tables 1, 2 and 3).

TABLE 2

Chemotherapy of subacute Trypanosoma rhodesiense *infection in guinea pigs with nitrofurazone (Furacin)*

Guinea Pig No.	Infection of G. Pig with *T. rhodesiense* Before Chemotherapy				Treatment with Single Dose Furacin, mg./kg., I.M.	Results Following Chemotherapy			Conclusion
	T. rhodesiense		Status of g. pig the day of therapy			Continuous presence of tryps. in blood	1st relapse of tryps. in blood (No. days)	Neg. micro. findings for tryps. (No. days)	
	Strain No.	No. syringe-passages in g. pigs	No. days infected	Tryp. in bl.					
1	A	31	32	+	50	0	14	—	Suppressive
2	B	20	31	+	50	0	8	—	Suppressive
3	D	30	31	+	50	0	14	—	Suppressive
4	F	22	31	+	50	0	16	—	Suppressive
5	H	8	31	+	50	0	14	—	Suppressive
6	A	33	33	+	75	0	—	365	Curative
7	B	19	34	+	75	0	22	—	Suppressive
8	D	29	31	+	75	0	5	—	Suppressive
9	F	21	31	+	75	0	9	—	Suppressive
10	H	12	32	+	75	0	31	—	Suppressive
11	A	9	55	+	100	+	—	—	Ineffective
12	A	28	31	+	100	0	67	—	Suppressive
13	B	10	63	+	100	0	11	—	Suppressive
14	B	18	31	+	100	0	10	—	Suppressive
15	C	9	35	+	100	+	—	—	Ineffective
16	D	7	20	+	100	0	32	—	Suppressive
17	D	38	38	+	100	0	16	—	Suppressive
18	F	20	32	+	100	0	22	—	Suppressive
19	G	6	40	+	100	+	—	—	Ineffective
20	H	6	35	+	100	0	—	365	Curative
21	A	29	31	+	125	0	17	—	Suppressive
22	B	25	33	+	125	0	16	—	Suppressive
23	D	27	31	+	125	0	10	—	Suppressive
24	F	19	31	+	125	0	18	—	Suppressive
25	H	5	32	+	125	0	26	—	Suppressive
26	A	30	32	+	150	0	—	365	Curative
27	B	26	32	+	150	0	39	—	Suppressive
28	D	26	31	+	150	0	16	—	Suppressive
29	F	24	32	+	150	0	84	—	Suppressive
30	H	4	31	+	150	0	35	—	Suppressive

Criteria of cure. Following administration of nitrofurazone, the peripheral blood of each guinea pig was examined microscopically daily (except Sundays) by the thick-drop stain method for trypanosomes.

The chemotherapy was considered *ineffective* when trypanosomes were continuously present in the peripheral blood of the guinea pig following administration of nitrofurazone and the animal died of trypanosomiasis.

The *suppressive* effect of the drug was manifested by total disappearance of trypanosomes from the circulating blood of each guinea pig within 24 hours after

TABLE 3

The comparative therapeutic effect of single dose of nitrofurazone on different strains of T. gambiense *and* T. rhodesiense *infections in guinea pigs*

Species of Trypanosomes		Results with Single Dose of Nitrofurazone mg./kg. I.M. per Guinea Pig		
Name	Strain	Ineffective	Suppressive	Curative
T. gambiense	E	—	50, 75	100, 125, 150
	K	—	—	100
	L	—	50, 100, 150	75, 100, 100, 125
	M	—	50, 75, 100, 150	—
	Q	—	100	—
	R	—	100	—
	T	—	100	—
	U	—	100	—
	X	—	50, 100	75, 125, 150
	Y	—	100	—
	Z	—	—	50, 75, 100, 125, 150
T. rhodesiense	A	100	50, 100, 125	75, 150
	B	—	50, 75, 100, 100, 125, 150	—
	C	100	—	—
	D	—	50, 75, 100, 100, 125, 150	—
	F	—	50, 75, 100, 125, 150	—
	G	100	—	—
	H	—	50, 75, 125, 150	100

administration of nitrofurazone, with reappearance of trypanosomes usually after three to five weeks following therapy (Tables 1 and 2). The animals in which relapse of trypanosomes was demonstrated all died subsequently of trypanosomiasis.

Nitrofurazone therapy was considered *curative* when the guinea pig recovered completely from the infection, i.e., trypanosomes disappeared from the circulating blood of the animal within twenty-four hours following therapy and were never seen again in daily subsequent examinations. Blood from guinea pigs which recovered from the infection was examined microscopically daily except Sunday for 200 to 369 days, and in the absence of trypanosomes these animals were considered cured and were discarded about a year following cessation of chemotherapy.

EXPERIMENTAL DATA

Chemotherapy of chronic T. gambiense *infection in guinea pigs.* The results of this study are summarized in Tables 1 and 3. Administration of a single dose of nitrofurazone, 50 to 150 mg./kg. body weight, produced variable therapeutic results in guinea pigs infected with different strains of *T. gambiense.*

Guinea pigs infected with *T. gambiense* strains E, K, X, or Z, treated with 100 to 150 mg./kg. of nitrofurazone one month following infection, were all cured. Administration of a reduced dose, 50 mg./kg. of the drug, produced suppressive effects in guinea pigs infected with strains E and X, and curative effects in a

guinea pig infected with strain Z. A single dose of the drug, 100 mg./kg., given intramuscularly to a guinea pig infected 130 days with *T. gambiense* strain X produced a suppressive effect.

Nitrofurazone therapy in guinea pigs infected with *T. gambiense* strain L produced variable results. Some of these animals were cured with a single dose, 75 to 125 mg./kg., of the drug, while in others, 50 to 150 mg./kg. produced a suppressive effect only.

Administration of a single dose of nitrofurazone, 100 mg./kg., to guinea pigs infected with the remaining six strains of *T. gambiense* (M, Q, R, T, U, and Y) produced only suppressive effects.

Out of 32 guinea pigs infected with *T. gambiense* and treated with nitrofurazone, suppressive effects were noted in 16 of the animals (50 per cent). Twenty-four hours following therapy trypanosomes disappeared from the circulating blood of these animals but reappeared in the blood again, usually 20 to 40 days following therapy. The earliest relapse in any guinea pig was 11 days and unusually late first relapses occurred in three guinea pigs 106, 119, and 328 days following cessation of therapy.

In the remaining 16 guinea pigs (50 per cent), curative results were obtained. Trypanosomes disappeared from the circulating blood of these animals 24 hours following therapy and were never found again although frequent microscopic examinations were made for periods ranging from 168 to 365 days. The complement-fixation tests, performed on blood samples of 10 of these guinea pigs 254 to 365 days following cessation of therapy, were invariably negative. All of these animals were discarded as cured.

Chemotherapy of subacute T. rhodesiense *infection in guinea pigs.* The results of this study are summarized in Tables 2 and 3. Administration of a single dose of nitrofurazone, 50 to 150 mg./kg., to guinea pigs infected with different strains of *T. rhodesiense* produced 10 per cent curative, 80 per cent suppressive, and 10 per cent ineffective results.

A single dose of nitrofurazone, 100 mg./kg., given to three guinea pigs infected with *T. rhodesiense* strain A, C, or G, failed to halt the course of infection. Trypanosomes were constantly present in the peripheral blood of these animals immediately following therapy, and the guinea pigs died of trypanosomiasis within 50 days, as did the untreated infected controls.

A single dose of the drug, 50 to 150 mg./kg., produced suppressive effects in 24 out of 30 guinea pigs infected with *T. rhodesiense.* On the day following therapy trypanosomes disappeared from the circulating blood, but reappeared again, usually within 5 to 26 days following cessation of therapy. Late relapses occurred after 67 and 89 days in two of the animals. Although the life span of these animals was prolonged as a result of nitrofurazone therapy, all the guinea pigs which relapsed succumbed eventually to trypanosomiasis.

Three infected guinea pigs treated respectively with 75, 100, and 150 mg./kg. of the drug in a single dose were apparently cured. Trypanosomes disappeared from the blood of these animals within 24 hours following treatment and could not be found by frequent microscopic examinations during 365 days after cessation of therapy.

DISCUSSION

The experimental data show that chronic *Trypanosoma gambiense* infection in guinea pigs can be cured or suppressed with a single dose of nitrofurazone of 100 to 150 mg./kg. given intramuscularly. Although a limited number of guinea pigs infected with different strains of *T. gambiense* were used in this study, a careful—almost daily microscopic search in the blood of these animals for trypanosomes revealed a therapeutic result which appears to be significant. As one might expect, there was a pronounced difference between the several strains of *T. gambiense* in their response to the same dose of the drug. With some strains nitrofurazone therapy produced 100 per cent cures, while with others the infections were either suppressed or 50 per cent were cured and 50 per cent suppressed (Tables 1 and 3). Guinea pigs in the early stage of *T. gambiense* infection (about 30 days) responded better to therapy than those treated in the later stages of the disease (130 to 216 days).

Because of the limited number of experiments, no conclusive evidence was found as to the comparative therapeutic effects of the drug on a given strain of *T. gambiense* in guinea pigs after different numbers of syringe-passages.

Packchanian (1955) has demonstrated that a single maximum therapeutic dose of nitrofurazone is less effective in acute *T. gambiense* infection in mice than when the same amount of the drug is administered daily for three to four days in divided doses.

This study was designed primarily to find the therapeutic effects of a single dose of nitrofurazone on chronic *T. gambiense* and subacute *T. rhodesiense* infection in guinea pigs. The same dose of nitrofurazone was apparently less effective on subacute *T. rhodesiense* infection than it was on chronic *T. gambiense* infection (Tables 1, 2 and 3). It appears that if the drug is administered in adequate dosage over a period of time (about a month) and if a desirable schedule for treatment is discovered, particularly before relapses of trypanosomes are expected, a higher percentage of cures may be obtained in both *T. gambiense* and *T. rhodesiense* infections. Such studies will be the subject of another communication.

Nitrofurazone therapy given orally in *T. gambiense* sleeping sickness in man has already shown very encouraging results (Evens, Niemegeers and Packchanian, 1957).

SUMMARY

Administration of single doses of nitrofurazone of 50 to 150 mg./kg. I.M. to 32 guinea pigs each infected with one of 11 different strains of *T. gambiense* cured half the infections and suppressed the rest. The effectiveness of a given dose of nitrofurazone varied with the strain of *T. gambiense*; infections caused by some strains (E, K, X, or Z) were cured, while infections caused by other strains (M, Q, R, T, U, and Y) were only suppressed. Variable therapeutic results were obtained with infections caused by strain L. Nitrofurazone produced a better therapeutic response in guinea pigs infected with *T. gambiense* when the drug was administered about a month following the infection than when treatment was initiated three to five months following infection.

Single doses of nitrofurazone were apparently not so effective in subacute *T. rhodesiense* infections as in chronic *T. gambiense* infections in guinea pigs. Single doses of nitrofurazone, 50 to 150 mg./kg. I.M., administered to 30 guinea pigs each infected with one of seven different strains of *T. rhodesiense* were curative in 10 per cent, suppressive in 80 per cent and ineffective in 10 per cent of the animals.

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NITROFURAZONE THERAPY OF TRYPANOSOMA GAMBIENSE SLEEPING SICKNESS IN MAN

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It is well known that many cases of African human trypanosomiasis relapse after treatment with various commonly used trypanocides such as Arsobal (Mel B, 3854 R.P.), Bayer 205 (Antrypol, Belganyl, Fourneau 309, Germanin, Moranyl, Naganol, Naphuride, Suramin), Lomidine (pentamidine isothienate), tryparsamide, etc. The prognosis in these drug-resistant cases is very poor and the mortality very high; hence, the need for a new drug that will cure not only new cases, but also those refractory to the present trypanocides.

Packchanian (1955) demonstrated the curative effects of nitrofurazone (Furacin)[1] on *Trypanosoma gambiense* and *Trypanosoma rhodesiense* infections in mice. The present communication reports the therapeutic effects of nitrofurazone on human patients suffering from African sleeping sickness. Initially, therapy was conducted only on a limited number of hopeless relapsing cases which were refractory to all known trypanocides, even after treatment for several years. As soon as it became evident that nitrofurazone therapy was effective in some of these cases, it was extended to additional severe relapsing cases with poor prognosis, as well as to a limited number of previously untreated cases with and without nervous involvement.

CLINICAL CASES

Thirty-two patients in various stages of *Trypanosoma gambiense* infection were selected for this study. All were native Africans who had apparently contracted the infection in southwestern Belgian Congo; they were kept under treatment and observation in a sanatorium known as the "Lazaret" at Leopoldville. For this study they were divided into five groups according to the severity of the disease or previous therapy with other trypanocides, and were given nitrofurazone either alone or in conjunction with Lomidine, or with Bayer 205, Lomidine and Arsobal, as will be described.

Group 1 consisted of three previously untreated adult patients in the early stages of the disease (Table 1, Cases 1, 2 and 3). Group 2 consisted of three previously untreated children in the nervous stage of the disease with *T. gambiense* present in the lymph nodes, the peripheral blood and the cerebrospinal fluid (Table 1, Cases 4, 5 and 6). Group 3 consisted of nine chronic relapsing cases, all refractory to previous intensive and prolonged treatment with trypanocides in current use (Table 2, Cases 7–15; Diagrams 1 and 2). Group 4 consisted of three patients from Group 3, Cases 13, 14 and 15, who relapsed after previous treatment with various trypanocides including nitrofurazone; these cases are

[1] Prepared by Eaton Laboratories, Norwich, New York.

TABLE 1

Chemotherapy of new cases of T. Gambiense *sleeping sickness with nitrofurazone alone or associated with Lomidine*

Clinical cases		Laboratory findings prior to chemotherapy					Chemotherapy				Laboratory findings after chemotherapy					Effects of therapy
		Trypanosomes present in			C.S.F.							Trypanosomes present in		C.S.F.		
Case No.	Weight in kg.	Lymph node aspirate	Thick blood smear	Spinal fluid (centrifuged)	Cells/cu. mm.	Protein gm./liter	Drug	Daily dosage mg./kg.	No. of days treated	Total drug admin. mg./kg.	No. of months after treatment	Thick blood smear	Spinal fluid (centrifuged)	Cells/cu. mm.	Protein gm./liter	
1	51.0	+	+	0	3.43	0.25	Nitrofurazone	9.8	1	186	¼	0	0	13.70	0.25	Questionable
				0	11.24	0.25		29.5	6							
2	57.0	+	+	0	1.87	0.25	Nitrofurazone	8.8	1	325	10	0	—	0.62	0.22	Favorable
				0	6.87	0.22		26.3	12							
3	50.0	+	+	0	1.25	0.22	Nitrofurazone	10.0	1	371	4	0	....	0.93	0.22	Favorable
				0	3.75	0.20		30.0	12							
4	16.0	+	+	+	12.81	0.25	Lomidine	4.0	6	24	2	0	+	50.60	0.30	Relapse
							Nitrofurazone	6.3	1							
								12.5	1	94						
								18.5	4							
5	15.0	+	+	+	57.40	0.40	Lomidine	4.0	6	24	16	0	0	0.62	0.16	Favorable
							Nitrofurazone	16.7	1							
								25.0	1	309						
								33.4	8							
6	9.5	+	+	+	950.00	0.88	Lomidine	4.0	6	24	11	0	0	1.87	0.20	Favorable
							Nitrofurazone	31.6	13	410						

designated as Cases 13*, 14* and 15* in Tables 3 and 4. Group 5 consisted of seventeen patients, hopeless relapsing cases with severe nervous involvement and a very poor prognosis, previously treated unsuccessfully for several years with various trypanocides other than nitrofurazone (Tables 3 and 4, Cases 16–32).

LABORATORY PROCEDURES

Standard laboratory procedures were used to establish the diagnosis of *T. gambiense* sleeping sickness in each patient prior to nitrofurazone therapy, and essentially the same techniques and procedures were utilized during the course of treatment and for several months following cessation of therapy in order to determine the effectiveness of the drug.

The presence or absence of *T. gambiense* in gland punctures (lymph node aspirates), thick blood smears and centrifuged cerebrospinal fluid was determined by microscopic examination occasionally supplemented by cultural tests. White cell counts of spinal fluid were determined by standard procedure. Protein in the spinal fluid was determined by the Sicard-Cantaloube method and results were recorded as grams protein per liter. Globulin in the spinal fluid was determined by the Weichbrodt tests and results recorded as positive or negative. The colloidal benzoin test (Guillain, Loroche and Lechelle) on spinal fluid was performed only in the first five tubes, the most important in this test for trypanosomiasis (00000).

The antigen used in the complement-fixation test on blood serum consisted of *Trypanosoma equiperdum* collected from rat blood at the height of infection. A strong positive reaction was recorded as ++++, a weak positive reaction as ++, a doubtful reaction as ± and a negative reaction as 0.

TREATMENT

Nitrofurazone (Furacin) 5-nitro-2-furaldehyde semicarbazone, is slightly soluble in water but readily absorbed from the intestinal tract. Tablets of 100, 250 and 500 mg. were used in this study. Since this is a new drug, the course of therapy was varied somewhat among different groups as indicated below. The selected dosage of the drug for a given day was administered orally in two, three, or four divided doses along with milk, bananas, or water.

In earlier studies it had been found that nitrofurazone when used alone in chronic, arsenic-resistant cases of sleeping sickness produced good initial results but only 22 per cent lasting cures (Table 2; Diagrams 1 and 2). However, in some instances, the use of two or more trypanocides concomitantly or in succession has shown apparent synergetic or tonic effects and has even reduced the toxicity of the drugs to the patient. For these reasons Lomidine, an effective and relatively nontoxic trypanocide in the peripheral blood, was used with nitrofurazone in the treatment of newly diagnosed cases in 3 small children in poor physical condition, with central nervous system involvement. Lomidine alone would never have cured these patients (Table 1).

For the treatment of the hopeless, relapsing, drug-resistant cases it was thought

TABLE 2

Effects of nitrofurazone therapy on chronic cases of African sleeping sickness refractory to previous treatment with various known trypanocides

Clinical cases			Previous treatment with trypanocides*	Laboratory findings prior to treatment with nitrofurazone				Nitrofurazone chemotherapy			Laboratory findings after treatment with nitrofurazone					Effects of therapy
				Trypanosomes in		C.S.F.						Trypanosomes in		C.S.F.		
Case No.	Age	Weight in kg.		Thick blood smear	Spinal fluid (centrifuged)	Cells/cu. mm.	Protein gm./liter	Daily dosage mg./kg.	No. of days treated	Total drug adm. mg./kg.	No. of days after treatment	Thick blood smear	Spinal fluid (centrifuged)	Cells/cu. mm.	Protein gm./liter	
7	21	53.0	T, M, E, A, L, Ms, B	0	+	174.9	0.50	18.9 28.4	12 12	556	8 42	0 0	0 +	19.60 —	0.25 —	Improved Relapsed
8	32	54.0	A, T, L, M, P	0	+	341.2	0.40	18.5 23.2 27.8 9.3 27.8	12 1 3 1 5	477	8 44	0 0	0 +	40.60 —	0.38 —	Improved Relapsed
9	3	16.0	Mo, T, M, L, A	0	+	164.9	0.50	15.6 31.2	1 15	485	8 381	0 0	0 0	9.90 0.93	0.38 0.20	Improved Cured
10	26	42.0	T, A, Ms	0	+	78.7	0.60	23.8 35.8	2 9	370	8 386	0 0	0 0	7.81 8.75	0.60 0.20	Improved Cured
11	15	31.0	T, M, A, L	0	+	429.8	0.45	8.1 24.2	1 25	613	8 332	0 0	0 +	8.43 —	0.55 —	Improved Relapsed
12	30	40.0	T, M, A, L, Mo	+	+	271.8	0.70	12.5 25.0 31.3 37.6	1 1 1 9	407	8 48	0 0	0 +	77.50 —	0.60 —	Improved Relapsed

13	14	43.0	A, Ms, T	0	+	110.1	0.40	11.6 17.4 23.2 29.1	1 7 3 6	378	8 66	0 0	0 +	31.80 —	0.45 —	Improved Relapsed
14	40	62.0	Ms, A, M, L, T	0	+	503.7	0.58	8.1 16.2 24.3	1 2 26	670	8 36	0 0	0 +	31.80 —	0.45 —	Improved Relapsed
15	Adult	27.0	Mo, Ms, A, B, M, L, A	0	+	98.7	0.50	18.5 27.8 37.0	3 2 15	667	8 180	0 0	0 +	24.60 98.70	0.38 0.50	Improved Relapsed

* Abbreviations of drugs: A = Arsobal (Mel B); B = Berenyl (Babesin); E = Emetic (Antimony potassium tartrate); L = Lomidine (Pentamidine metasulfonate); M = Moranyl (Bayer 205, Germanin, Suramin); Mo = Melarsen oxide; Ms = MFS-Friedheim (MSbB, Melarsen BAL); P = Puromycin (Stylomycin); T = Tryparsamide.

that combining nitrofurazone with Bayer 205, Lomidine and Arsobal might increase the percentage of cures. Bayer 205 and Lomidine are highly effective on *T. gambiense* in the peripheral blood. Although both of these drugs are ineffective on the typical nervous stage of the disease, they influence more or less the trypanosomes in the cerebrospinal fluid. A combination of these two drugs is less toxic than either preparation alone; both remain active in the body for a long time and "make life difficult for trypanosomes". Arsobal, which is highly effective as a trypanocide in the peripheral blood and for treating the nervous phase of the disease, is the most toxic of all trypanocidal agents, and is used chiefly for treating cases with nervous involvement in areas where tryparsamide-resistant strains of trypanosomes are common. It kills a high percentage of trypanosomes even in cases in which it has been used before and which have relapsed. The therapy used in each group of patients was briefly as follows:

1) *Treatment with nitrofurazone alone* was administered to Group 1, three previously untreated adult patients in the early stages of the disease, each of whom received 8.8 to 30 mg./kg. daily for 7 to 13 days; and to Group 3, nine chronic relapsing patients, who were given 8.1 to 37.6 mg./kg. daily for 11 to 29 days (Tables 1 and 2).

2) *Treatment with nitrofurazone in conjunction with Lomidine.* Group 2, three previously untreated cases (children) with nervous involvement, were treated simultaneously with Lomidine (4.0 mg./kg. intramuscularly daily or every other day for six days) and nitrofurazone (6.3 to 33.4 mg./kg. orally daily for six to eight days) (Table 1).

3) *Treatment with nitrofurazone in conjunction with Bayer 205, Lomidine and Arsobal.* Groups 4 and 5, consisting of twenty hopeless, relapsing cases of *T. gambiense* sleeping sickness were treated with the four trypanocidal drugs, a combination designated for convenience the "cocktail treatment" (C.T.). The following schedule (sequence and amount of the different drugs) constitutes one course of C.T. for an adult patient; nitrofurazone on the days indicated was administered in three divided doses, varying from 5.3 to 22.6 mg./kg.

1st day	Bayer 205	2 gm. I.V.
2nd–4th	Lomidine	4 mg./kg. I.M./daily/3 days
5th day	Arsobal	3.6 mg./kg. I.V.
	nitrofurazone	1–3 gm. orally
6th day	nitrofurazone	1–3 gm. orally
7th day	Arsobal	3.6 mg./kg. I.V.
	nitrofurazone	1–3 gm. orally
8th day	nitrofurazone	1–3 gm. orally
9th day	Arsobal	3.6 mg./kg. I.V.
	nitrofurazone	1–3 gm. orally
10th–13th	nitrofurazone	1–3 gm. orally/daily/4 days

This initial course of C.T. was followed immediately by a second course of similar composition and sequence. In some cases a third course was instituted

TABLE 3

Laboratory findings prior to cocktail treatment on chronic relapsing cases

Clinical cases			Trypanosomes present in			Cerebrospinal fluid				Blood
Case No.	Weight in kg.	Previous treatment with trypanocides†	Thick blood smear	Spinal fluid (centrifuged)	Hemoculture	Cells/cu. mm.	Protein gm./liter	Globulin Weichbrodt test	Colloidal benzoin test	Complement fixation test
13*	43	A, Ms, T, F	0	+	—	110.10	0.40	++	22333	±
14*	62	Ms, A, M, L, T, F	0	+	0	406.20	0.52	+++	33333	±
15*	30	Mo, Ms, A, B, M, L, F	0	+	—	98.70	0.50	+++	33333	++++
16	58	T, M, E, A, L	0	+	0	190.00	0.38	++++	23333	++++
17	80	A	0	+	+	384.90	1.00	+++	23333	++++
18	51	Ms, B, T, A, M, L	0	+	—	108.70	0.44	+++	23333	±
19	65	Ms, A, M, L	0	+	—	168.70	0.65	++++	11333	++
20	36	?, T, M, A	0	+	—	293.70	0.45	+++	23333	++
21	72	A, T	0	+	—	61.20	0.50	++	23333	±
22	28	A	0	+	0	163.70	0.38	+	22323	++++
23	50	M, T, E, A	0	+	—	151.80	0.65	++++	33333	—
24	60	A	0	+	—	247.40	0.38	++++	22333	++++
25	39	M, T, E, A	0	+	—	429.70	0.42	+++	33333	0
26	68	A	0	+	—	625.00	0.60	++	12330	±
27	46	T, D, M, A, L, E	0	+	—	53.74	0.18	+++	12331	±
28	51	Mo	0	+	0	229.90	0.40	++++	12333	++
29	85	A	0	+	0	95.00	0.20	+++	23331	0
30	55	T, M, E, A	0	+	—	234.90	0.24	+++	33333	++
31	58	A	0	+	—	113.70	0.22	++++	23333	++
32	62	A	0	+	—	640.00	0.28	+	33330	0

† Abbreviations of drugs: A = Arsobal; B = Berenyl; E = Emetic; F = Furacin (Nitrofurazone); L = Lomidine; M = Moranyl (Bayer 205); Mo = Melarsen oxide; Ms = MFS-Friedheim; T = Tryparsamide.

after an eight-day rest period, followed in three patients by a fourth course without any rest period (Tables 3 and 4).

Case-study following treatment. The therapeutic effects of nitrofurazone alone or in conjunction with other drugs were determined by following patients for several months to a year after cessation of chemotherapy. While all laboratory tests were performed on each patient at frequent intervals, only the most essential representative data appear in this report in order to conserve space. Criteria of cure were based chiefly on the total disappearance of *T. gambiense* from the blood and cerebrospinal fluid, reduction of high cell counts in the spinal fluid to within normal range, negative complement-fixation tests and definite clinical improvement.

RESULTS OF CHEMOTHERAPY

Chemotherapy of new, previously untreated cases with nitrofurazone alone (Group 1). This group consisted of three adult patients in the early stages of the disease

TABLE 4

Laboratory findings after cocktail treatment on chronic relapsing cases

Patient		Chemotherapy		Number of days after treatment	Trypanosomes present in		Cerebrospinal fluid				Blood	Results
Case No.	Weight in kg.	No. cocktail courses	Total nitrofurazone adm. in grams		Thick blood smear	Spinal fluid (centrifuged)	Cells/cu. mm.	Protein gm./liter	Globulin Weichbrodt test	Colloidal benzoin test	Complement fixation test	
13*	43	4	35.7	425	0	+	120.00	0.24	+++	12333	0	Relapsed
14*	62	4	57.5	120	0	+	98.60	0.38	+	11230	++	Relapsed
15*	30	2	27.0	180	0	+	53.60	0.40	+	2233?	++	Relapsed
16	58	3	51.5	306	0	0	2.18	0.40	++	11121	—	Favorable
17	80	3	52.5	8	0	0	16.20	0.52	+++	12333	±	?
18	51	3	21.0	365	0	0	4.06	0.20	0	00200	0	Favorable
19	65	2	35.5	365	0	0	2.50	0.20	+	00000	0	Favorable
20	36	2	22.5	8	0	0	26.80	0.50	++	—	++++	?
21	72	2	33.0	245	0	0	2.81	0.40	+	00100	0	Favorable
22	28	3	24.5	365	0	0	1.25	0.22	0	00000	±	Favorable
23	50	3	25.5	180	0	+	172.40	0.50	+++	23332	0	Relapsed
24	60	3	33.5	365	0	0	6.87	0.22	+	00000	±	Favorable
25	39	2	28.5	92	0	+	104.90	0.20	++++	33331	±	Relapsed
26	68	4	73.0	122	0	0	2.50	0.22	0	01200	0	Favorable
27	46	2	42.5	92	0	0	2.50	0.30	+	00320	—	Favorable
28	51	2	62.5	92	0	0	17.40	0.40	+++	23332	—	Favorable
29	85	2	57.0	30	0	0	12.50	0.15	++	01200	—	?
30	55	2	45.0	61	0	0	7.81	0.40	+	12320	0	Favorable
31	58	2	37.0	8	0	0	19.37	0.20	++	11330	±	?
32	62	2	45.0	61	0	0	52.50	0.30	+	11310	0	Favorable

(Table 1, Cases 1, 2 and 3). *T. gambiense* was demonstrated in the lymph nodes and peripheral blood but not in the cerebrospinal fluid. Cell counts, protein, globulin and colloidal benzoin reactions of the spinal fluid all were within normal range, but complement-fixation tests on blood samples were strongly positive.

Case 1 received orally a total of 186 mg./kg. nitrofurazone over a period of seven days. One week later this patient's condition was markedly improved, his blood and spinal fluid were free of trypanosomes and the complement-fixation test gave a doubtful reaction. This case failed to return for follow-up studies.

Cases 2 and 3 were treated orally with a total of 352 mg./kg. and 371 mg./kg. of the drug respectively over a period of 13 days. Both patients showed marked clinical improvement following therapy. Laboratory studies ten and four months respectively after cessation of therapy revealed no trypanosomes in the blood of either patient; the complement-fixation tests on the blood of these patients were weakly positive; and both showed a favorable prognosis as a result of adequate nitrofurazone therapy.

Nitrofurazone and Lomidine therapy in new, previously untreated cases with central nervous involvement (*Group 2*). This group consisted of three children

(Table 1, Cases 4, 5 and 6). Laboratory studies revealed the presence of *T. gambiense* in the lymph nodes, peripheral blood and spinal fluid. The cell count, globulin, and proteins of CSF of patients five and six were markedly increased. Colloidal benzoin test for patient four was 23313 and for patient five 23333.

Case 4 (weight 16 kg.) was treated over a six day period with a total of 94 mg./kg. nitrofurazone and daily injections of 4 mg./kg. of Lomidine. This patient did not respond to treatment and *T. gambiense* was demonstrated in the cerebrospinal fluid two months after cessation of therapy. This unfavorable result was due, in all probability, to inadequate nitrofurazone dosage.

Case 5 (weight 15 kg.) was given a total of 309 mg./kg. nitrofurazone over a ten day period, together with 4 mg./kg. Lomidine daily for six days. Sixteen months later the patient's blood and spinal fluid were free of trypanosomes. The cell count, globulin, protein and colloidal benzoin tests of CSF had dropped from initially high to normal levels, and the complement-fixation test on blood was negative. The therapy was, therefore, considered effective.

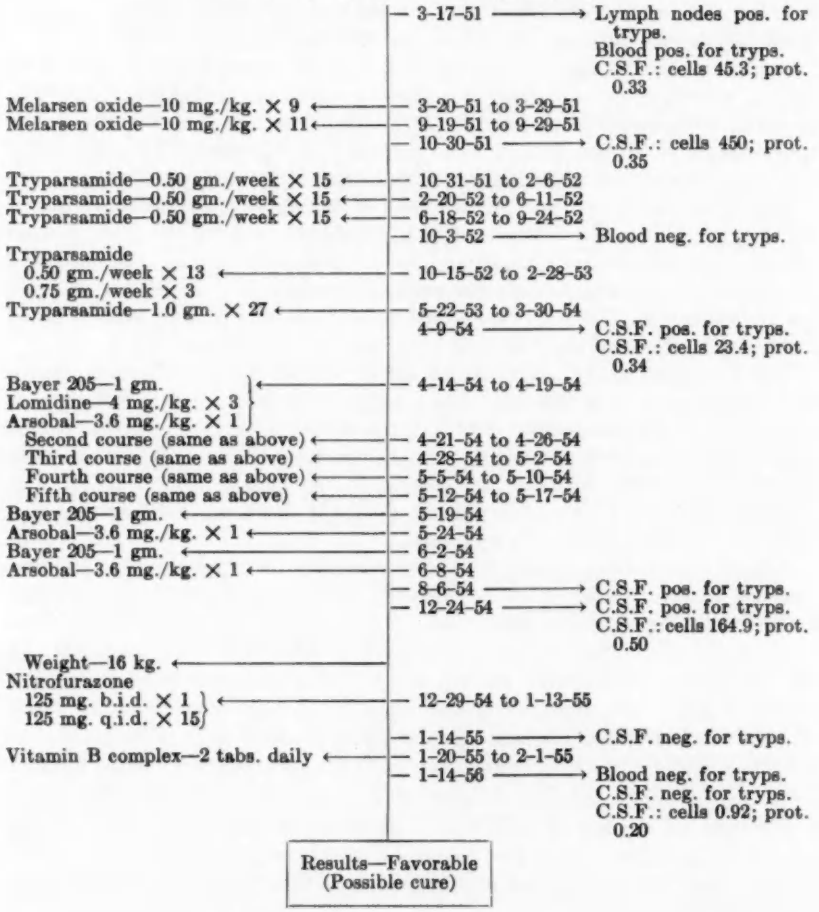
Case 6 (weight 9.5 kg.) received a total of 410 mg./kg. nitrofurazone over a period of thirteen days together with 4 mg./kg. Lomidine every other day for six injections. Eleven months following cessation of therapy, the blood and spinal fluid of this patient were free of trypanosomes and the cell count, protein and globulin of CSF had dropped from an initial high to normal levels. The complement-fixation test on blood was negative at this time. This case was also considered to have a favorable prognosis.

Nitrofurazone therapy alone on chronic relapsing cases refractory to previous treatment with various trypanocides (Group 3). This group of nine patients (Table 2, Cases 7 through 15) consisted of chronic cases of sleeping sickness with severe involvement of the central nervous system and with very poor prognosis. All had been treated with several courses of 3 to 7 different trypanocidal drugs for periods of one to five years. Diagrams 1 and 2 are representative of the types of drugs used, schedule of administration and laboratory findings pertaining to blood and spinal fluid before and after each course of treatment. While most of these drugs produced temporary suppressive effects, they failed to halt the course of infection. All of these patients had *T. gambiense* in their spinal fluid and the cell counts, globulin, protein and colloidal benzoin tests of the CSF were markedly increased. They were considered hopeless at the time nitrofurazone therapy was instituted.

These cases were treated orally with nitrofurazone for 11 to 29 days, the total daily dosage ranged from 18 to 30 mg./kg. administered in 3 or 4 divided doses, and each patient received a total of 370 to 670 mg./kg. All showed marked improvement eight days after therapy as evidenced by the disappearance of trypanosomes from the spinal fluid and a pronounced decrease of elevated cell counts; however, parasitologic relapse was demonstrated in the spinal fluid in all of these patients except in Cases 9 and 10. Trypanosomes appeared after cessation of nitrofurazone therapy within two months in five of the patients (Cases 7, 8, 12, 13 and 14), within six months in one (Case 15), and after nearly one year in another (Case 11), who had had rather high cell counts continuously

DIAGRAM 1

Case No. 9, 3 yr. old male, Weight—14.5 kg.

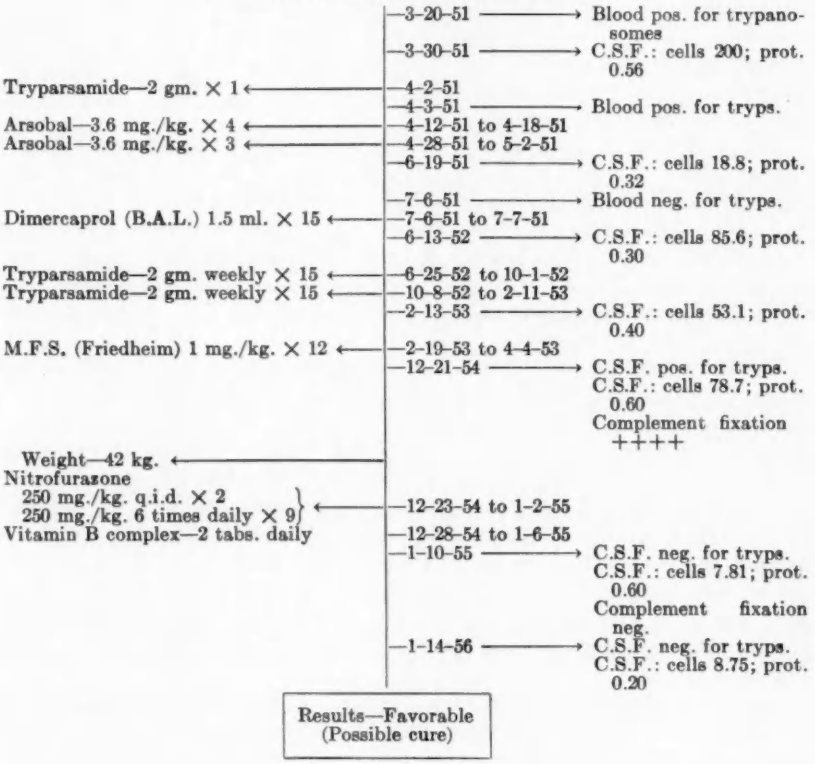

3-17-51 → Lymph nodes pos. for tryps. Blood pos. for tryps. C.S.F.: cells 45.3; prot. 0.33
Melarsen oxide—10 mg./kg. × 9 ← 3-20-51 to 3-29-51
Melarsen oxide—10 mg./kg. × 11 ← 9-19-51 to 9-29-51
10-30-51 → C.S.F.: cells 450; prot. 0.35
Tryparsamide—0.50 gm./week × 15 ← 10-31-51 to 2-6-52
Tryparsamide—0.50 gm./week × 15 ← 2-20-52 to 6-11-52
Tryparsamide—0.50 gm./week × 15 ← 6-18-52 to 9-24-52
10-3-52 → Blood neg. for tryps.
Tryparsamide 0.50 gm./week × 13 0.75 gm./week × 3 ← 10-15-52 to 2-28-53
Tryparsamide—1.0 gm. × 27 ← 5-22-53 to 3-30-54
4-9-54 → C.S.F. pos. for tryps. C.S.F.: cells 23.4; prot. 0.34
Bayer 205—1 gm. Lomidine—4 mg./kg. × 3 Arsobal—3.6 mg./kg. × 1 ← 4-14-54 to 4-19-54
Second course (same as above) ← 4-21-54 to 4-26-54
Third course (same as above) ← 4-28-54 to 5-2-54
Fourth course (same as above) ← 5-5-54 to 5-10-54
Fifth course (same as above) ← 5-12-54 to 5-17-54
Bayer 205—1 gm. ← 5-19-54
Arsobal—3.6 mg./kg. × 1 ← 5-24-54
Bayer 205—1 gm. ← 6-2-54
Arsobal—3.6 mg./kg. × 1 ← 6-8-54
8-6-54 → C.S.F. pos. for tryps.
12-24-54 → C.S.F. pos. for tryps. C.S.F.: cells 164.9; prot. 0.50
Weight—16 kg. ←
Nitrofurazone 125 mg. b.i.d. × 1 125 mg. q.i.d. × 15 ← 12-29-54 to 1-13-55
1-14-55 → C.S.F. neg. for tryps.
Vitamin B complex—2 tabs. daily ← 1-20-55 to 2-1-55
1-14-56 → Blood neg. for tryps. C.S.F. neg. for tryps. C.S.F.: cells 0.92; prot. 0.20
Results—Favorable (Possible cure)

for five months preceding the relapse. One patient (Case 12) who had had trypanosomemia in addition to flagellates in the spinal fluid at the beginning of therapy, died of complete exhaustion from the disease some weeks after nitrofurazone treatment.

Two patients in this group were apparently cured by nitrofurazone therapy: Case 9, a three year old child who received 485 mg./kg. nitrofurazone over a 16 day period; Case 10, a twenty-six year old female, the mother of Case 9, who received a total of 370 mg./kg. nitrofurazone over a period of 11 days. Following cessation of therapy both patients steadily improved. Their spinal

DIAGRAM 2

Case No. 10, 26 yr. old female, Weight—44 kg.


3-20-51 → Blood pos. for trypanosomes
3-30-51 → C.S.F.: cells 200; prot. 0.56
Tryparsamide—2 gm. × 1 ←
4-2-51
4-3-51 → Blood pos. for tryps.
Arsobal—3.6 mg./kg. × 4 ←
Arsobal—3.6 mg./kg. × 3 ←
4-12-51 to 4-18-51
4-28-51 to 5-2-51
6-19-51 → C.S.F.: cells 18.8; prot. 0.32
7-6-51 → Blood neg. for tryps.
Dimercaprol (B.A.L.) 1.5 ml. × 15 ←
7-6-51 to 7-7-51
6-13-52 → C.S.F.: cells 85.6; prot. 0.30
Tryparsamide—2 gm. weekly × 15 ←
Tryparsamide—2 gm. weekly × 15 ←
6-25-52 to 10-1-52
10-8-52 to 2-11-53
2-13-53 → C.S.F.: cells 53.1; prot. 0.40
M.F.S. (Friedheim) 1 mg./kg. × 12 ←
2-19-53 to 4-4-53
12-21-54 → C.S.F. pos. for tryps. C.S.F.: cells 78.7; prot. 0.60 Complement fixation ++++
Weight—42 kg. ←
Nitrofurazone
250 mg./kg. q.i.d. × 2
250 mg./kg. 6 times daily × 9 ←
12-23-54 to 1-2-55
Vitamin B complex—2 tabs. daily
12-28-54 to 1-6-55
1-10-55 → C.S.F. neg. for tryps. C.S.F.: cells 7.81; prot. 0.60 Complement fixation neg.
1-14-56 → C.S.F. neg. for tryps. C.S.F.: cells 8.75; prot. 0.20
Results—Favorable (Possible cure)

fluid was free of trypanosomes for a year as indicated by frequent microscopic examination. Cell counts in the spinal fluid decreased to within normal range, and both cases showed a continuing evolution toward cure (Table 2, Diagrams 1 and 2).

Effects of nitrofurazone in conjunction with Bayer 205, Lomidine and Arsobal on chronic cases refractory to trypanocides including nitrofurazone (Group 4). Three cases of African sleeping sickness from Group 3, which had relapsed after treatment for several years with various trypanocides including nitrofurazone, were given the "cocktail treatment" (Tables 3 and 4, Cases 13*, 14* and 15*). The condition of these three patients markedly improved following C.T.; the trypanosomes disappeared from the spinal fluid and the abnormally high cell counts and protein levels came down to nearly normal limits. However, trypanosomes reappeared in the CSF in all three of these patients in 425, 120 and 180 days respectively after cessation of therapy.

Effects of cocktail treatment on chronic relapsing cases refractory to trypanocides other than nitrofurazone (*Group 5*). This group consisted of seventeen hopeless chronic cases with severe nervous involvement, high cell counts, and very poor prognosis (Tables 3 and 4, Cases 16–32). Trypanosomes were demonstrated microscopically in the spinal fluid of all these patients but not in thick blood smears; however, blood cultures made on five of these patients revealed one positive for flagellates (Case 17).

Ten of these cases received two courses of C.T.; the total nitrofurazone per patient (apart from Bayer 205, Lomidine and Arsobal) varied from 460 to 1,222 mg./kg. and the individual dosage varied from 8.7 to 22.6 mg./kg. t.i.d. for 18 days. Six patients received three courses of C.T.; the total nitrofurazone per patient varied from 440 to 490 mg./kg. and the individual dosage ranged from 5.3 to 11.0 mg./kg. t.i.d. for 27 days. Only one patient received four courses of C.T.; the total nitrofurazone administered was 1,080 mg./kg. in single doses of 10 mg./kg. t.i.d. for 36 days.

The early response to C.T. was very favorable in all of the patients in this group. Trypanosomes disappeared promptly from the spinal fluid and as a rule the high cell counts dropped to within normal levels. Two of the patients (12 per cent) showed recurrence of trypanosomes and a high cell count in the spinal fluid three and six months following cessation of therapy. Six other patients (35 per cent) of this group who showed favorable prognosis were not followed long enough to determine the final outcome of the disease. The remaining nine patients (53 per cent) of the group probably were cured by C.T., as frequent microscopic examinations of their spinal fluid from 92 to 365 days following cessation of therapy were all negative for trypanosomes and the cell counts, protein, globulin, and colloidal benzoin remained within the normal range (Tables 3 and 4).

Side effects of nitrofurazone therapy. Nausea and vomiting occurred in three patients during nitrofurazone administration and were not alleviated to any extent even when the drug was given with milk or bananas; however, these effects were transient and in two cases subsided before termination of therapy.

Rather serious muscular and arthritic pains occurred in some of the patients about ten days after the beginning of nitrofurazone therapy. These varied in intensity from slight symptoms to pain so severe that they could hardly walk even with the aid of a cane. One patient complained of pain in his fingertips and diminished tactile sensation, and showed some incoordination of finger movements. Some of these patients were given vitamin B complex (4 tablets daily for 8 to 14 days) and recovered from these side effects several days sooner than those not so treated. None of these patients showed any objective physical findings except slightly increased patellar reflexes. All of the above side effects subsided within a month after cessation of therapy and did not return.

DISCUSSION

Only four trypanocidal drugs, i.e., Bayer 205, Lomidine, Arsobal and tryparsamide have been used extensively in recent years for treating African sleeping

sickness in man, with varying degrees of success. Each of these has certain advantages and disadvantages in the therapy of human trypanosomiasis. Nitrofurazone can now be added to this group as a nontoxic trypanocide with a rather wide spectrum for human therapy of African sleeping sickness.

Nitrofurazone is not only effective on trypanosomes in the circulating blood but, of even more significance, it penetrates the "blood-brain barrier" and is also effective against trypanosomes in the cerebrospinal fluid. Since therapy with this drug is exclusively *per os*, large groups of patients can be treated in a short time. The drug is stable in warm climates, even when stored for long periods.

Nitrofurazone blood levels can be readily determined by measurement of its absorption in the ultraviolet spectrum and by bacteriological assay methods. In experimental animals definite and prolonged blood levels do occur after a single oral administration of nitrofurazone (200 mg./kg.). Peak concentration in the blood (0.6 mg. %) is usually reached eight hours after oral administration and diminishes considerably after 24 hours. Distribution of the drug in the whole blood is estimated to be ⅔ in the cells and ⅓ in the plasma.

It was established also that nitrofurazone does penetrate the "blood-brain barrier," and that the drug levels in the cerebrospinal fluid can also be assayed. One gram nitrofurazone was given orally to a dog (40.98 mg./kg.); two hours after administration of the drug examinations of serum and spinal fluid of the animal revealed 0.75 mg. per cent of the drug in serum and 0.22 mg. per cent in the cerebrospinal fluid (unpublished data).

Definite and prolonged nitrofurazone blood levels occur also in human patients, but no attempt was made in this study to determine or maintain a given blood level. Such studies are, however, highly desirable and special emphasis in the clinical trial of nitrofurazone should be given to dosage and blood levels of the drug, and to intervals of treatment in order to establish the most effective standard therapeutic procedure in the various types of African sleeping sickness, particularly those refractory to all other known trypanocides.

It is recommended that nitrofurazone be used not only alone but in refractory cases in conjunction with other trypanocides. In the cases here presented, a combination of these drugs with nitrofurazone yielded a fairly high percentage of lasting cures in patients with chronic infection which had proved to be incurable by Bayer 205, Lomidine or Arsobal alone (Tables 3 and 4).

Most of the patients in this study are still under observation and the final clinical and laboratory results pertaining to these and other cases will be the subject of another communication.

SUMMARY

Nitrofurazone, 5-nitro-2-furaldehyde semicarbazone (Furacin), a new trypanocidal drug, was used for the first time on human beings in the treatment of 32 patients suffering with *Trypanosoma gambiense* sleeping sickness, with encouraging therapeutic results. Administered orally, the drug is readily absorbed from the intestinal tract and is effective against trypanosomes in the circulating blood and in the cerebrospinal fluid. Oral doses of 2.1 to 12.5 mg./kg. t.i.d. for

seven to thirty-six days were tolerated by the patients without any permanent ill effects. During therapy some cases experienced side effects of temporary nausea and vomiting, and muscular and articular pains in the legs, which subsided within a month following cessation of therapy.

Cures were effected with nitrofurazone alone in 2 of 3 adults without central nervous system involvement; 2 of 3 children with serious C.N.S. involvement were cured with a combination of nitrofurazone and Lomidine. A so-called "cocktail treatment" of nitrofurazone in conjunction with Bayer 205, Lomidine and Arsobal produced favorable prognoses in 9 of 20 hopeless relapsing cases refractory to all previous treatment. This combination was no more toxic than any of the drugs taken separately.

In treating African sleeping sickness, particularly chronic, drug-resistant cases with severe nervous involvement, it is recommended that nitrofurazone be used orally, either alone or in conjunction with other trypanocides.

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EFFICACY OF AMODIAQUIN HYDROCHLORIDE (CAMOQUIN HYDROCHLORIDE) AGAINST GIARDIASIS[1]

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At the Jewish Hospital of Brooklyn routine stool examinations are made on nursing and food-handling personnel and if pathogens, including *Giardia lamblia*, are found, they may not be employed until stool examinations are negative. Persons infected with *G. lamblia* are usually asymptomatic and there is no absolute proof of the parasite's pathogenicity, but many clinicians believe that the parasite should be removed by specific treatment. In the case of the hospital employees, this is a requirement. For a number of years atabrine, 0.1 gm. three times daily for 3 to 5 days, has been standard treatment. This dosage sometimes, though rarely, produces toxic effects such as yellowish discoloration of the skin, nausea, vomiting or abdominal pain.

Recently the use of amodiaquin has been reported in the treatment of giardiasis. Mein (1951), using the drug for malaria treatment in Brazil, found that it was effective in eliminating *Giardia*. Konar (1953), in India, used amodiaquin as a one-dose treatment in 28 cases of giardiasis and found it to be nontoxic and highly effective, curing 21 after one dose, and 7 more after a second dose. Lamadrid-Montemayor (1954), in Mexico, reported that amodiaquin was 100 per cent successful in 50 cases of giardiasis after one course, there being no toxic effects. He and Konar used 0.6 gm. amodiaquin in a single dose.

At the Jewish Hospital of Brooklyn we recently studied the use of amodiaquin in the treatment of giardiasis, giving 0.6 gm. in one dose with water after breakfast. After treating 15 patients we discontinued this form of therapy because we could not duplicate the results reported by the observers mentioned above. Out of a total of 15 patients treated, only 5 were cleared of the infection after one course of amodiaquin. Four patients received a second dose and of these three were cleared of their infection but were lost to adequate follow-up. One person still harbored the parasite after a second dose. Thus the cure rate after one dose was 33 per cent. Of these 15 subjects, 11 were hospital personnel and asymptomatic. The remaining four subjects were ward or clinic patients being treated and/or studied for chronic urticaria, pain in the lower portion of the abdomen, intermittent constipation and diarrhea, and gallbladder disease respectively. In none could the complaints be directly ascribed to their infection with *Giardia lamblia*.

The diagnosis of giardiasis was established in each case by stool examination. We used wet preparations in saline and iodine and the concentration method of Loughlin and Spitz (1949).

[1] The Camoquin hydrochloride used in this study was supplied by Parke, Davis and Company, Detroit.

COMMENT

Our results indicating that a single 0.6 gm. dose of amodiaquin is relatively ineffective against giardiasis do not confirm the study of Lamadrid-Montemayor (1954) who reports a 100 per cent cure rate. It is possible that a larger single dose might be more effective but the drug may be toxic in such doses. There is no real advantage over atabrine if repeated doses have to be taken. We have resumed using atabrine as the specific therapy of giardiasis. Atabrine in the recommended dose over such a short course (3 to 5 days) rarely has toxic effects.

SUMMARY

Giardiasis, even when asymptomatic, is a public health problem in hospital personnel. With the aim of avoiding the occasional toxic effects of atabrine, amodiaquin was administered in a single dose of 0.6 gm. to 15 adults harboring *Giardia lamblia*, resulting in the cure of only 5, or 33 per cent.

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ISOLATION OF YELLOW FEVER VIRUS FROM *HAEMAGOGUS LUCIFER, H. EQUINUS, H. SPEGAZZINII FALCO, SABETHES CHLOROPTERUS* AND *ANOPHELES NEIVAI* CAPTURED IN PANAMA IN THE FALL OF 1956

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The staff of the Gorgas Memorial Laboratory has been interested for a number of years in the vectors of yellow fever in Panamá. In the fall of 1949 an attempt was made to isolate virus from mosquitoes captured at two locations; Pacora, where human yellow fever deaths were recorded in November and December, 1948 and Buena Vista, where deaths occurred in August and September, 1949. From the end of September, 1949 to the onset of the dry season in early January, 1950, 2,723 mosquiotes were collected from the Pacora station and 1,672 from the Buena Vista station. However, no yellow fever virus was recovered (Rodaniche, 1951). Although no further human cases were recorded from eastern Panamá until the present year, there was reason to suspect the infection had remained endemic there. This view was supported by the finding of occasional juvenile monkeys with high titers of neutralizing antibodies against yellow fever in their blood (Rodaniche, in press). In August, 1956 a new attempt to recover the virus was made.

The first mosquito collecting station was established in August 1956 in the San Blas Territory, in the Sangandi River Valley eight kilometers due south of the Mandinga air strip on the Atlantic side of the Continental Divide. Methods employed in setting up this type of station have been described by Galindo, Trapido and Carpenter (1950). Shortly after collections were begun at Mandinga, the first human yellow fever death in Panamá since the 1948–1951 outbreak was verified. The source of infection was traced to the headwaters of the Pacora River, in the Cerro Azul area, some 60 km. east of the Canal Zone and 20 km. southwest of the Mandinga collecting site on the Pacific side of the Continental Divide. A second station was then established in this area, where collections were made from September 13 to October 9 when they were discontinued for the remainder of the year.

METHODS

Methods employed were essentially the same as those described by Rodaniche and Galindo (1957) in their report on the vectors of yellow fever in Guatemala. Mosquitoes were macerated in 10 per cent rhesus serum saline containing 1,000 Units of penicillin and 1,000 micrograms of streptomycin per cubic centimeter. After centrifugation, the supernatant fluid was used to inoculate groups of 6 young adult white Swiss mice by the intracerebral route. Occasionally groups of infant mice also were used. Yellow fever virus was identified by cross-immunity tests in which mice surviving inoculation with the mosquito viral isolates

were challenged with Theiler's French neuroadapted virus and conversely mice previously immunized against the French strain were challenged with the virus strains recovered from mosquitoes. Mouse protection tests also were conducted using a known positive and known negative monkey serum. In the current investigation mosquitoes were held in the field at atmospheric temperatures for one day before being packed in ice for shipment, a procedure not found feasible in Guatemala. This was done in order to permit digestion of any freshly ingested blood.

RESULTS

In Table 1 data are presented concerning the collections made at the Mandinga station during the 4 month period from August 22 to December 20, 1956. A total of 7,094 mosquitoes comprising 15 different species or species groups were collected and tested. The most abundant species was *Aedes leucocelaenus clarki*, but large numbers of *Haemagogus lucifer*, *spegazzinii falco* and *Sabethes chloropterus* also were found. All four of these species showed heavier density in September than in the succeeding months. Only one isolation of yellow fever virus was made, from a pool of 137 *H. lucifer* collected between September 1 and 6. This strain was recovered initially in mice but later the residue of the original mosquito suspension which had been stored in dry ice was injected subcutaneously in a normal rhesus monkey. The animal did not die but developed fever and showed circulating virus followed by the appearance in its

TABLE 1

Yellow fever virus isolations from mosquitoes captured at the Mandinga station from August 22 to December 20, 1956 and injected into mice

Species of mosquito	Total no. collected	Nos. collected monthly				
		Aug.	Sept.	Oct.	Nov.	Dec.
H. spegazzinii falco	853	86	335	170	137	125
H. equinus	258	19	50	63	74	52
H. lucifer	1,162 (1)	87	540 (1)	237	203	95
A. leucocelaenus clarki	1,707	160	655	485	320	87
S. chloropterus	1,409	162	624	367	158	98
*Sabethes spp.**	654	61	256	179	77	81
Wyeomyia spp.	460	178	14	175	55	38
Trichoprosopon magnum	153	40	0	34	41	38
Trichoprosopon spp.	155	28	23	104	0	0
Anopheles neivai	142	7	24	16	87	8
Anophelini	46	1	0	14	27	4
Psorophora spp.	49	15	0	12	7	15
Aedes spp.	12	3	3	0	1	5
Mansonia spp.	27	8	0	7	8	4
Culex spp.	7	2	0	0	0	5
Total	7,094 (1)	857	2,524	1,863	1,195	655

Number in parenthesis represents virus isolation.

* Not including *S. chloropterus*.

TABLE 2

Yellow fever virus isolations from mosquitoes captured at the Cerro Azul station from September 13 to October 9, 1956 and injected into mice

Species of mosquito	Total no. collected	Nos. collected monthly		Virus isolations
		Sept.	Oct.	
H. spegazzinii falco	56	23	33	1
H. equinus	48	21	27	1
H. lucifer	649	328	321	2
Aedes leucocelaenus clarki	10	7	3	0
S. chloropterus	265	188	77	2
Sabethes spp.	44	28	16	0
Wyeomyia spp.	298	144	154	0
Anopheles neivai	49	4	45	1
Total	1,419	743	676	7

* Not including *S. chloropterus*.

blood of high titers of neutralizing antibody against the French neuroadapted strain.

In Table 2 are listed data concerning the collections made at Cerro Azul during a 26 day period, from September 13 to October 9, 1956, when collections were temporarily suspended. A total of 1,419 mosquitoes were captured and tested, comprising 8 different species or species groups. The most abundant forms were *H. lucifer*, *S. chloropterus* and *Wyeomyia spp.* Seven isolations of yellow fever virus were made from five different species. Virus was recovered once from a pool of 48 *H. equinus*, once from a pool of 56 *H. spegazzinii falco* and once from a pool of 49 *Anopheles neivai*. Each pool included all specimens collected during the 26 day period. Virus was recovered twice from *H. lucifer*, first from a pool of 133 specimens collected from September 9 to 20, and again from a pool of 107 mosquitoes collected between September 21 and 26. Two isolations were also made from *S. chloropterus* pools of 114 and 51 specimens, captured from September 21 to October 2 and from October 5 to 15 respectively. The shortest incubation period observed in mice during this study was 6 days and the longest 14 days with an average of 9.5 days. Two strains showed sufficient initial virulence to kill all mice injected that were not sacrificed for further study. The other five strains required one or more additional intracerebral passages before they produced uniformly fatal infections.

DISCUSSION

H. spegazzinii falco has long been recognized as an important natural vector of yellow fever in South America. The literature concerning this species was recently reviewed by Whitman (1951). Rodaniche and Galindo (1957) reported the first isolations of the virus from *Sabethes chloropterus* and pure pools of *H. equinus* captured in Guatemala in 1956. In the present report the first recoveries of yellow fever virus from pure pools of *H. lucifer* and from *Anopheles neivai*

are described. Boshell and Osorno-Mesa (1944) recovered virus from a mixed pool of *H. spegazzinii falco, lucifer* and *equinus* in Columbia but it is not known which species was incriminated. An attempt was made by Galindo, Rodaniche and Trapido (1956) to transmit yellow fever by bite using a Panamanian strain of *H. lucifer*. This experiment gave an unsatisfactory result due to death of all but one of the infected mosquitoes before the end of the incubation period. It was uncertain whether the one remaining mosquito fed or merely probed. However, virus was recovered from a large proportion of the mosquitoes by mouse injection after incubation periods varying from 14 to 30 days. The finding of 3 pools of *H. lucifer* positive for yellow fever, one from the Atlantic side and two from the Pacific side, indicates the importance of this species here. It is very common in the forested areas of eastern Panamá near the Canal Zone (Galindo, Trapido and Carpenter, 1950) and its period of peak incidence seems to bear a direct relationship to the appearance of jungle yellow fever here.

The status of *Anopheles neivai* is more obscure. The present isolation is the first from this genus of mosquito. Davis and Shannon (1931) attempted experimental transmission with two South American species of *Anopheles*, *A. albitarsis* and *tarsimaculatus* but failed to infect monkeys by exposing them to the bites of these anophelines or by injection of suspensions of the ground mosquitoes after an incubation period of 12 to 18 days. Further experimentation is needed to determine whether *A. neivai* can harbor virus for long periods of time and whether it transmits by bite.

It is of interest that naturally infected specimens of *Aedes leucocelaenus clarki*, a geographical race of a recognized South American vector, were not found in the course of this investigation. This was the predominant species at Mandinga where yellow fever activity was apparently minimal. Only 10 specimens were captured at Cerro Azul where virus activity was high, an inadequate sample.

CONCLUSIONS

Yellow fever virus was isolated once from 7,094 mosquitoes captured near Mandinga on the Atlantic side of the Continental Divide east of the Canal. This isolation was made from *H. lucifer*. It was isolated 7 times from 1,419 mosquitoes captured at Cerro Azul on the Pacific side, 60 kilometers east of the Canal Zone. Two recoveries each were made from *H. lucifer* and *S. chloropterus*, and one recovery each from *H. spegazzinii falco*, *H. equinus* and *Anopheles neivai*.

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ISOLATION OF ILHÉUS VIRUS FROM *SABETHES CHLOROPTERUS* CAPTURED IN GUATEMALA IN 1956

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In 1956 Rodaniche reported the first isolation of Ilhéus (Ilhéus encephalitis) virus in Central America and reviewed the literature. The virus was recovered from a pool of mosquitoes of the genus *Psorophora*, including principally *P. ferox* but also *P. lutzii* and *P. varipes*. In the present publication a brief report will be made of the isolation of this virus from *Sabethes chloropterus* captured in Guatemala in 1956. This finding is of interest because it represents the first isolation in Guatemala and the first from this species of mosquito.

The mosquitoes were captured by Drs. J. Boshell and G. Bevier of the Pan American Sanitary Bureau in the Motagua valley of Guatemala during the 1956 outbreak of jungle yellow fever there, and forwarded to the Gorgas Memorial Laboratory where they were classified. The principle objective of the investigation was the isolation of yellow fever virus, and the recovery of the Ilhéus virus was an incidental finding. Methods employed in this study have been described in the publication of Rodaniche and Galindo (1957) and need not be repeated here.

A total of 10,251 mosquitoes were received from March through November of 1956 (Table 1). Ilhéus virus was isolated only once, from a pool of 48 *Sabethes chloropterus* captured on April 9 and 10 in the Motagua valley. Four of the six young adult white mice injected intracerebrally with a suspension of these mosquitoes sickened and died or were sacrificed after an incubation period of 15 to 17 days. By the 4th passage the incubation period had shortened to 4 days, with death of all mice injected within 7 days, and by the 8th passage part of the mice were showing slight symptoms in 3 days. Symptoms were tremors, ataxia, spasmodic movements, paralysis, coma and death. Cross immunity experiments and mouse protection tests with a known negative and known positive yellow fever serum showed the virus to be distinct from that of yellow fever. Final classification was based on immunological comparison with the Honduran strain of Ilhéus virus previously mentioned.

Fifteen adult white mice were immunized against the Guatemalan strain and then challenged by the intracerebral injection of approximately 10^{-5} LD/50 of the Honduran strain. All survived throughout a 30-day observation period whereas the entire control group sickened and died within 6 days. In reverse cross-immunity experiments, two groups of 12 mice each, previously immunized against the Honduran strain, were challenged by the intracerebral injection of approximately 10^{-5} LD/50 of the Guatemalan virus in its 8th and 15th mouse passages respectively. There were no deaths among the test animals whereas all controls died in 6 to 7 days. Mouse protection tests also were carried out using pooled hyperimmune mouse serums prepared against each of the two strains.

TABLE 1

*Classification of mosquitoes received from the Motagua Valley of Guatemala from March through November, 1956**

Species or species group	Total
Haemagogus equinus	1,711
Haemagogus mesodentatus	4,175
Sabethes chloropterus	3,304 (1)†
Sabethes spp.‡	293
Aedes spp.	32
Psorophora cingulata	2
Psorophora ferox	7
Psorophora lutzii	369
Trichoprosopon spp.	27
Wyeomyia spp.	289
Mansonia spp.	3
Anopheles spp.	3
Chagasia spp.	36
Total	10,251 (1)

* Captures were made by Dr. J. Boshell and G. Bevier of the P.A.S.B.

† Number in parenthesis represents virus isolation.

‡ Not including *S. chloropterus*.

The serum prepared against the Honduran strain neutralized 3.3 log units of homologous virus and 2.6 log units of the heterologous strain respectively. The immune serum prepared against the Guatemalan strain neutralized 2.4 log units of homologous virus and 2.3 of the heterologous strain.

The isolation of Ilhéus virus from mosquitoes captured in Guatemala indicates that this virus was active there simultaneously with yellow fever, although apparently activity was minimal. The capacity of *S. chloropterus* to harbor Ilhéus virus is of especial interest as the same species was also found to harbor yellow fever virus by Rodaniche and Galindo (1957). No isolations were made from a total of 378 *Psorophora*, the genus implicated in previous isolations of this virus in Brazil, Trinidad and Honduras.

CONCLUSIONS

The Ilhéus virus was isolated from a pool of 48 *Sabethes chloropterus* captured in the Motagua Valley of Guatemala in 1956. This isolation represents the first from Guatemala and the first from this species of mosquito.

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THE ISOLATION OF ST. LOUIS VIRUS FROM TRINIDAD MOSQUITOES[1]

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During 1953–54 an island-wide survey of immunity to certain viruses in residents of Trinidad, B.W.I. revealed the presence of antibodies neutralizing the St. Louis encephalitis virus in 15 of 348 sera. In three instances the serum contained antibodies to St. Louis virus and to none of eight other viruses tested (Downs *et al.*, 1956).

Commencing in April 1955, an intensive search for viruses in arthropods was undertaken in several localities. Over 160 thousand specimens (largely mosquitoes) were ground and inoculated into infant mice. Besides a number of as yet unidentified agents which were isolated, three strains of St. Louis virus were obtained from pools of mosquitoes. These mosquitoes were captured biting human beings in forested areas. This article presents the details of the isolation and identification of these viruses.

ISOLATION

Strain # 1 (TRVL Specimen # 9355). A collection of 530 mosquitoes caught at dusk 5 September 1955 in the Arena Forest (1½ miles southeast of San Rafael), was sorted into 15 pools and inoculated into infant mice. From one pool, consisting of eight *Culex* (*Culex*) *coronator*, a virus was isolated.

The mosquitoes were ground with alundum in a mortar and suspended in one milliliter of diluent.[2] The suspension (Specimen # 9355) was then centrifuged for one half hour in an angle centrifuge at about 10,000 rpm in a 4° C. cold room. The supernatant fluid was inoculated intracerebrally into a group of two-day-old mice. A passage of brain to two-day-old and adult mice was subsequently made from a mouse suspiciously ill on the eighth postinoculation day.[3] None of the adult mice of this passage group died or became ill. One of the baby mice died on the third and another on the eighth postinoculation day. A passage of brain

[1] The studies and observations upon which this paper is based were conducted with the support and under the auspices of the Government of Trinidad and Tobago, the Colonial Development and Welfare Scheme and The Rockefeller Foundation.

[2] The diluent is composed of 0.12 molar sodium chloride; 0.006 molar monobasic sodium phosphate; 0.012 dibasic sodium phosphate; and 0.75% fraction 5 bovalbumin. The pH is adjusted to between 6.8 and 7.3 by the addition of 0.3 molar sodium hydroxide solution. Sterilization is effected by filtration through a Seitz EK pad. Crystalline penicillin G and streptomycin sulphate are added to produce a final concentration of 400 units per milliliter of the former and 250 micrograms per milliliter of the latter.

[3] A portion of the brain suspension from this first passage was stored in the dry ice box. Because of the peculiar behavior of the first passage, the suspension was re-inoculated into four groups of two-day-old mice. All of these mice sickened by the third day and were dead by the fifth day; the virus was obtained from several passages from these mice.

was made from a third mouse which sickened on the sixth day. This passage produced a fatal infection in a group of adult mice as well as a group of two-day-old mice. The baby mice were all dead by the fourth postinoculation day and the adults by the fifth. The agent passed easily through a bacteria-tight Seitz EK pad.

Strain #2 (TRVL Specimen #9404). In a collection of 2,680 mosquitoes and 121 *Culicoides* processed as 25 pools and inoculated into infant mice, two virus strains were isolated. One of these has yet to be identified; the other is the agent (TRVL #9404) under discussion, which was isolated from a pool of 70 *Culex* (*Melanoconion*) *caudelli* collected on the ground at the "monkey tree" in the Melajo Forest 7 September 1955. This tree station, some 7½ miles north of Sangre Grande, is the one at which yellow fever virus was isolated from a howler monkey and repeatedly from *Haemagogus* mosquitoes in 1954 (Downs *et al.*, 1955).

The pool of 70 *caudelli* was ground in a mortar and suspended in two milliliters of diluent. Following centrifugation of the suspension (TRVL #9404) at about 10,000 rpm for one half hour at 4°C., the supernate was inoculated intracerebrally into a group of two-day-old mice. Some of the mice were sick on the sixth postinoculation day, at which time a passage of brain from one of them was made into a group of two-day-old and a group of adult mice. The remaining mice were all dead by the eighth postinoculation day. The agent was easily established in adult mice in the first passage. Filtration of the virus through a bacteria-tight Seitz EK pad was accomplished with a second passage brain suspension.

Strain #3 (TRVL Specimen #9464). From the same collection site in the Melajo Forest two days later, 9 September 1955, 2,123 mosquitoes were processed as 18 pools. Two agents were again isolated; one, stemming from a pool of 374 *Psorophora* (*Janthinosoma*) *ferox*, represents the third St. Louis isolate to be reported.

This *Psorophora* pool was ground in a mortar and suspended in three milliliters of diluent. Following centrifugation as above, the suspension (Specimen #9464) was inoculated intracerebrally into a group of two-day-old mice. A passage of brain was made from one of the sick mice on the sixth postinoculation day. The remaining mice were all dead by the eighth postinoculation day. The agent was established in adult mice in the first passage. A brain suspension prepared from the first-passage mice successfully passed through a bacteria-tight Seitz EK pad.

No differences were observed in the clinical picture produced by these strains in adult and infant mice inoculated intracerebrally. The incubation period in baby and adult mice was slightly less than three days, and most mice were dead by the fifth day. The disease was characterized by the usual manifestations, beginning with signs of irritation, convulsions, prostration and paralysis.

At the time of inoculation of the mosquito suspensions, an aliquot was stored in a dry ice box in a sealed glass ampoule. When these strains were isolated, as well as all of the others isolated during this period in 1955 and through most of

1956, the dry ice storage facilities of the laboratory were inadequate for the large amount of material being inoculated. Arrangements were therefore made for storing the arthropod suspensions elsewhere. Unfortunately, this did not prove as satisfactory as was hoped and the suspensions were known to have been thawed from time to time. When the unsatisfactory nature of this arrangement was realized, ampoules were removed to a more reliable storage place as soon as virus was suspected of being present. Although attempts to re-isolate the viruses from the present three suspensions were none of them successful, a careful examination of the records has revealed no information that would cast doubt on the validity of the isolations. Further, no known strains of St. Louis virus had been brought into the laboratory.

IDENTIFICATION

Each virus was tested in an intracerebral neutralization test with specific immune sera containing antibodies to the following viruses: St. Louis, Semliki Forest, Bunyamwera, Eastern, Western, and Venezuelan equine encephalomyelitis, yellow fever and Ilhéus. The results of these tests indicated that the three agents were most closely related to St. Louis virus.

Immune sera were prepared to each virus by inoculating rabbits, intraperitoneally, with 0.5 ml. of 10^{-4} dilution of infected mouse brain of a given strain. A month later the animals were exsanguinated and the serum collected. Each rabbit had been bled several times from the ear vein prior to inoculation in order to accumulate sufficient serum to serve as a control for the corresponding immune serum. All sera were stored at −20° C. in sterile lusteroid tubes. The neutralizing capacity of each serum for each of the three viruses was determined by titration of the virus simultaneously in the pre- and postinoculation serum. The tests were set up by mixing equal volumes of untreated serum and suitable virus dilutions and incubating the mixtures for an hour at 37°C. Each dilution was inoculated intracerebrally into a group of adult mice. The results of these tests are summarized in Table 1. The results have been expressed as the log MLD_{50} of virus neutralized by the immune serum as compared to the preinoculation control serum from the same animal. The endpoints were calculated by the

TABLE 1

Cross neutralization of 9355, 9404, and 9464 immune rabbit sera by homologous and heterologous strains

Sera	Virus strains		
	9355	9404	9464
9355 Immune	4.0*	3.5	2.3
9355 Immune	2.9	2.7	2.1
9404 Immune	2.8	3.1	2.7
9404 Immune	3.4	3.8	3.0
9464 Immune	2.7	2.3	3.6
9464 Immune	2.7	2.6	3.6

* The results are expressed as the logarithms of the MLD_{50}'s of virus neutralized.

method of Reed and Muench (1938). It can be seen that no significant difference exists between the immune sera in regard to their neutralizing capacity. In this respect, therefore, the viruses have been considered to be either very closely related or identical.

Hemagglutination antigens to each of the viruses (9355, 9404, 9464) were prepared from small groups of two-day-old mice inoculated intracerebrally with infected brain suspensions, according to the directions recommended by Casals and Brown (1954). The optimum condition for hemagglutination by each of the antigens was found to be the same, namely, a pH of 7.0 at 24° C. The antigen from strain 9464 was tested in a hemagglutination-inhibition test. Homologous antiserum and sera known to inhibit hemagglutination by Group B viruses inhibited hemagglutination by 9464. This same antigen also fixed complement well in the presence of homologous antiserum and of known St. Louis virus antisera sent to this laboratory from the Rockefeller Foundation Laboratories in New York.

As it was undesirable to bring known strains of St. Louis virus into Trinidad until the presence of the virus could be definitely established, two of the strains, 9404 and 9464, were sent to the Rockefeller Foundation Laboratories. There, Dr. Leslie Spence studied the relationship of the Trinidad strains to the Parton strain of St. Louis encephalitis virus by means of the hemagglutination-inhibition and complement-fixation tests.

The hemagglutination-inhibition tests were carried out according to the technique described by Casals and Brown (1954). The inhibition of the 9404 and 9464 hemagglutinins by various Group B mouse immune and hyperimmune sera—St. Louis, West Nile (Egypt 101 strain), Ilhéus, and Japanese B—was compared with the inhibition by the same sera of the St. Louis virus hemagglutinin. In some of the tests other Group B hemagglutinins, such as yellow fever, Japanese B and Ilhéus, were included. The 9404 and 9464 strains reacted with the sera in the same manner as did the St. Louis strain and the other Group B hemagglutinins. Although the hemagglutination-inhibition test does not serve as a specific diagnostic test for viruses of the "B" Group, the results of these tests did establish the strains 9404 and 9464 as members of Group B.

TABLE 2

Results of complement fixation tests with the 9404, St. Louis, Ilhéus, and Japanese B virus antigens and their immune sera

Antigens	Mouse hyperimmune sera							
	9404		St. Louis		Ilhéus		Jap. B	
	Serum titer	Antigen titer	Serum titer	Antigen titer	Serum titer	Antigen titer	Serum titer	Antigen titer
9404	128*	256	128	256	32	16	32	8
St. Louis	128	256	128	512	—	—	—	—
Ilhéus	16	32	—	—	128	256	—	—
Jap. B	16	32	—	—	—	—	128	128

* The results are expressed as the reciprocal of the highest dilution of serum or antigen, as the case may be, giving a three or four plus fixation.

The 9404 strain was compared with St. Louis (Parton strain), Ilhéus and Japanese B viruses in the complement-fixation test. The antigens and mouse hyperimmune sera were prepared by the method of Casals and Brown (1954). The homologous and heterologous serum and antigen titer (box titration) were obtained in a series of cross-complement fixation tests. The results of these tests have been summarized in Table 2. It can be seen from these results that the titers of both sera and antigens are highest with the St. Louis and 9404 strains, indicating that these two strains are more closely related to each other than the 9404 strain is to either the Ilhéus or Japanese B strains of virus. The same conclusion was drawn from a similar test comparing the 9464, St. Louis and West Nile (Egypt 101 strain) viruses.

SUMMARY AND CONCLUSIONS

St. Louis encephalitis virus has been isolated from three ground pool-breeding species of mosquitoes collected in Trinidadian forests. Identity was established by comparison with a known strain of St. Louis virus and other related viruses. These represent the first strains of St. Louis virus known to be isolated from mosquitoes outside of the continental United States.

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THE ISOLATION OF ST. LOUIS VIRUS FROM A NESTLING BIRD IN TRINIDAD, BRITISH WEST INDIES[1]

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St. Louis encephalitis virus (SLE) has thus far been encountered only in the United States. Collier *et al.* (1953, 1956) describe the occurrence of complement-fixing antibodies in the sera of some persons suffering from encephalitis and in sera of healthy individuals in Surinam. Verlinde *et al.* (1955) describe the occurrence of neutralizing antibodies against SLE in sera of residents of Curaçao infected with encephalitis. Downs *et al.* (1956) describe the occurrence of neutralizing antibodies in healthy residents of Trinidad. Owing to possibilities of cross-immune reactions, however, such serological data alone must be viewed with some reserve.

In 1955, SLE virus was isolated three times from mosquitoes captured in forested regions of Trinidad (Anderson *et al.*, 1957). This is the first time this virus has been isolated from mosquitoes south of the continental limits of the United States. The present paper reports the isolation of SLE virus (TRVL 13375) from a nestling bird in Trinidad.

A 10-day-old nestling of *Leptoptila v. verreauxi* (rusty dove) was collected on August 21, 1956 at Vega de Oropouche. The nestling was very weak, and expired in the hand. A blood specimen of one half cubic centimeter was taken immediately, and diluted with physiological saline containing antibiotics. The final serum dilution was estimated as 1:5. The blood specimen was brought refrigerated into Port-of-Spain, centrifuged, and the serum inoculated intracerebrally into two-day-old mice. The mice sickened as a group on the seventh postinoculation day. Two were sacrificed for passage, and the remainder taken for preparation of a brain antigen. The passaged virus was shown to pass the Seitz EK filter readily, and to be pathogenic for both infant and weaned mice on intracerebral inoculation. When virus dosages in the range of 100–1000 MLD_{50} were inoculated, the average survival time for baby mice and for adult mice was four–five days. An aliquot of the original serum specimen which had been stored at −60°C. for 85 days was reinoculated for confirmation, and titered in tenfold dilutions, groups of six weaned mice being used for each dilution. The virus titer was calculated according to the method of Reed and Muench (1938) and was found to be 1043 MLD_{50} per milliliter of undiluted serum.

A hemagglutinating and complement-fixing antigen was prepared according to the method of Casals and Brown (1954) using mouse brains of four baby mice from the litter inoculated with the original serum specimen and harvested on the seventh postinoculation day.

[1] The studies and observations upon which this paper is based were conducted with the support and under the auspices of the Government of Trinidad and Tobago, the Colonial Development and Welfare Scheme and The Rockefeller Foundation.

In hemagglutination testings, this antigen showed optimum hemagglutination at a pH of 6.8 and worked well at temperatures of 24° and 37°C. These results were confirmed with a second batch of antigen. Hemagglutination by the antigen was inhibited by Group B reactive immune sera.

In complement-fixation tests using the same antigen, and St. Louis immune rabbit serum in a "box" titration using several antigen dilutions against several serum dilutions with two units of complement in the test, the antigen titered out to 1:64 and the immune serum to 1:64 as limits of full fixation of complement. No reaction was observed with Ilhéus immune rabbit serum at antigen and antiserum dilutions of 1:8.

Immune serum was prepared in two rabbits by intraperitoneal inoculation of a 10^{-6} dilution of infected mouse brain (2,560 MLD_{50}), one dose only, and bleeding 30 days later. When this immune serum was tested in complement-fixation tests using antigens prepared from Ilhéus (Brazilian strain), dengue (Trinidad 1751 strain), yellow fever (French neurotropic strain) and St. Louis (Trinidad 9464 strain) viruses, and also from the unknown (homologous) virus, the strongest reactions were obtained in the "box" titrations using St. Louis virus and the homologous virus. Ilhéus virus gave a lesser degree of complement-fixation, but not as much as with its homologous antiserum. Dengue was only weakly reactive, and yellow fever non-reactive. These tests were extended in the Rockefeller Foundation Virus Laboratories in New York, including tests with St. Louis (Parton strain), Japanese B (Nakayama strain) and West Nile (Egypt 101 strain) antigens. The close relationship to St. Louis was confirmed. Data are summarized in Table 1.

Tests were also run in the New York Laboratories using mouse hyperimmune sera collected after six injections of virus suspension. These sera were tested in

TABLE 1

Identification of 13375 virus complement fixation tests using several antigens and rabbit immune serum against several viruses

(Titers are highest titer of antigen and antiserum showing complete fixation of complement)

Antigens	Rabbit antisera prepared against							
	13375 Rabbit #1		13375 Rabbit #2		9464 St. Louis*		Ilhéus	
	Antigen	Serum	Antigen	Serum	Antigen	Serum	Antigen	Serum
13375	1:64	1:32	1:128	1:32	1:64	1:64	0	0
St. Louis	1:16	1:32	1:32	1:32	1:32	1:32	1:2	1:8
Ilheus	1:16	1:8	1:16	1:16	—	—	1:32	1:16
Dengue	1:4	1:4	1:4	1:4	—	—	—	—
Japanese B	1:32	1:16	1:32	1:16	—	—	—	—
West Nile	1:32	1:8	1:32	1:16	—	—	—	—

Symbols: — indicates no test performed.

0 indicates no complement fixation at antigen and serum dilutions of 1:8.

* This serum was prepared by two inoculations of St. Louis virus intraperitoneally at intervals of 10 weeks and serum was collected 31 days after the second inoculation.

TABLE 2

Identification of 13375 virus by complement fixation tests using several antigens and mouse hyperimmune serum against several viruses

(Titers are highest titer of antigen and antiserum showing complete fixation of complement)

Antigen	Mouse hyperimmune sera prepared					
	St. Louis		Japanese B		West Nile	
	Antigen	Serum	Antigen	Serum	Antigen	Serum
13375	*1:256**	1:128	*1:32*	1:32	*1:32*	1:16
St. Louis	*1:256*	1:128	—	—	—	—
Japanese B	—	—	*1:256*	1:128	—	—
West Nile	—	—	—	—	*1:128*	1:32

* Titers of antigens have been italicized to bring out the similarity in titers when identical systems are used (St. Louis:13375); and the difference when only related systems are used (13375:Jap. and 13375:West Nile).

TABLE 3

Identification of 13375 virus by neutralization of virus by various normal and immune rabbit sera

TRVL Specimen No.	Identity of serum	Virus titration	MLD_{50} of virus neutralized by immune serum
10355-5	Normal	$10^{-3.5}$	—
10355-11	St. Louis immune	$10^{-4.3}$	17000
7269-3	Ilhéus immune	$10^{-6.6}$	79
3110-2	Yellow fever immune	$10^{-7.0}$	35
	Bovalbumin diluent control	$10^{-3.2}$	—

"box" complement-fixation tests against homologous (13375), St. Louis (Parton), Japanese B (Nakayama) and West Nile (Egypt 101) antigens and the results are summarized in Table 2. The close similarity of TRVL 13375 to St. Louis is evident.

An identification test was performed by intracerebral inoculation of weaned mice with serial tenfold dilutions of the unknown virus in rabbit immune serum specimens prepared against St. Louis, Ilhéus and yellow fever viruses. Virus titrations were calculated and compared with a control titration of virus in normal rabbit serum. Table 3 presents these data.

The two antisera prepared by inoculation of rabbits with TRVL 13375, as mentioned earlier, were also tested against the homologous virus in neutralization test. Immune serum of one rabbit protected against 25,000 MLD_{50} of homologous virus and that of another rabbit, against 3,160 MLD_{50}. When these same immune sera were tested against St. Louis virus (strain TRVL 9464), each serum neutralized approximately 100 MLD_{50} of the virus.

DISCUSSION

Hemagglutination and complement-fixation tests indicate that the agent isolated is a member of the Group B complex of arthropod-borne (Arbor) viruses,

which includes such agents as dengue, St. Louis, Ilhéus, yellow fever, West Nile, Ntaya, Murray Valley, Japanese B encephalitis, Uganda S and Zika viruses. Complement-fixation test data indicate that the agent is very similar to a standard St. Louis virus (Parton strain), while there is enough difference in the titers both of antigens and sera to set it apart from Japanese B, West Nile, Ilhéus, dengue and yellow fever viruses. Neutralization tests indicated a close relationship to St. Louis virus.

SUMMARY

St. Louis encephalitis virus has been recovered from the serum of a nestling bird in Trinidad, B.W.I. This is the first time this agent has been recovered from a local, nonmigratory bird south of the continental limits of the United States.

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ANTIBODIES TO POLIOMYELITIS VIRUS AND SOCIOECONOMIC FACTORS INFLUENCING THEIR FREQUENCY IN CHILDREN IN ISRAEL[1]

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The new tissue culture techniques initiated by Enders *et al.*, 1949, have made it possible to determine in vitro, with relative ease and without great expense, the presence of a large number of viruses and their specific antibodies. It was decided to apply these methods to a study of the excretion of intestinal viruses in a child population in Israel, and at the same time to determine the presence of antibodies against poliomyelitis virus in these individuals. The present paper reports the distribution of antibodies in the population studied and an evaluation of various extraneous factors which may influence the time of their appearance.

MATERIALS AND METHODS

Blood samples were collected from children living in Kiryath Yovel, a new housing project on the southwestern outskirts of Jerusalem. The population with which this study is concerned is the new immigrant community of Kiryath Yovel, which comprised in June, 1955, 452 families with an average of slightly more than four persons per family. The families, all of whom have immigrated to Israel since 1948 (predominantly during 1949–51), come from some thirty different countries, which may be grouped as European, North African and Middle Eastern, giving the following percentage distribution of families by area of origin of the head of the family: 42 per cent from Europe, 36 per cent from North Africa and 22 per cent from Middle Eastern countries. Generally speaking, it is a population of large families with a high birth rate (36 per 1,000), a low infant mortality (15 per 1,000 live births) and a high proportion of children, especially among families originating from North Africa. The economic level of the community is low, the majority of adult males being unskilled workers; at the time of this study, some 20 per cent of the families were receiving aid, in one form or another, from the Municipal Welfare Department.

The community is housed in cement buildings, each containing two, four or eight flats. The flats consist of 1½, 2½ or (rarely) 3 rooms, the majority having a total floor area of 30–35 square metres. All houses have water-borne sewerage, electricity and a clean municipal water supply, but the general state of cleanliness of the area is not always satisfactory.

A combined preventive and curative health service is provided to the community by a family health center, organised by the Hadassah Medical Organization and situated in the area. Medical help is easily available, and a close contact

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is maintained between the centre and the families it serves, particularly those families which include young children.

It was planned to obtain a random sample of approximately one half of all children of preschool age by visiting every alternate home in which there were children, the arrangement of families in the area being quite haphazard. Since some of the children visited were not in the house at the time or (less frequently) it was for some reason not possible to take blood from them, blood was in fact obtained from about one third of all children up to the age of six years. As far as it is possible to obtain a random sample of so small and heterogenous a community, this group of children may be considered as such. In the case of older children, blood was obtained from a fifth of the children aged 6–7 years and from less than 10 per cent of children aged 8–12 years. These children were chosen from among those attending summer school and clearly do not represent a random sample of the total child population at these ages. The total sample comprises 155 children aged from 6 months to 12 years. Their distribution by place of birth of the father was as follows: 73 per cent from North Africa (predominantly Morocco), 19 per cent from Middle Eastern countries, and 8 per cent from European countries.

Venous blood was obtained from these 155 children during June and July 1955. The blood samples were centrifuged within twenty four hours and stored in rubber stoppered test tubes at −20°C. until tested.

Viruses. The Mahoney, MEFI and Saukett strains as representative strains of types I, II and III of poliomyelitis virus were obtained from Dr. P. Lepine's Laboratory at the Pasteur Institute, Paris; they were repeatedly checked for their identity with the aid of monkey antisera obtained from Dr. J. L. Melnick, New Haven, Conn. The strains were propagated in tissue cultures of human cells as described below. For neutralization tests several larger pools were prepared and stored at −20°C. The pools were repeatedly titrated before the tests and a complete titration was also run with each neutralization experiment.

Tissue cultures. At the beginning of this study tissue cultures were prepared from human embryonic lung tissue, but the majority of the tests were carried out in trypsinised human amniotic cells which proved a uniformly sensitive medium (Zitcer *et al.*, 1955). After confluent growth had been obtained the cultures were kept in a medium containing 0.5% lactalbumin hydrolysate in Earle solution to which 2% calf serum was added. Virus titers were calculated by the method of Reed and Muench (1938) using 4 tubes per virus dilution. For the neutralization tests 100 $TCID_{50}$ were employed. The sera were inactivated for 30 minutes at 56°C. before the test and used at a dilution of 1:4, in Earle solution; 0.2 ml. of the serum dilutions were mixed with 0.2 of the appropriate virus dilution and the mixtures kept at room temperature for 1 hour. They were then transferred to an ice-water bath until inoculated, in 0.1 ml. amounts, into each of two tissue cultures containing 1 ml. medium. After 4 days the tubes were examined microscopically. In tubes where neutralization occurred the cell sheet was undamaged, whereas in serum cell mixtures which did not contain specific antibodies and in the virus control tubes a clear cytopathogenic effect was

TABLE 1

Presence of antibodies against poliomyelitis virus in sera of 155 children

Antibodies present against type		Number of sera in age groups							
		6–12 months	1	2	3	4	5	6–7	8–12 years
I	No.	1	8	16	27	17	14	20	14
	%	17	33	70	84	89	87	100	93
II	No.	0	2	11	18	11	13	20	14
	%	0	8	47	56	58	81	100	93
III	No.	5	11	13	25	17	13	19	14
	%	83	46	57	78	89	81	95	93
I + II	No.	0	2	6	12	7	4	1	3
or II + III	%	0	8	26	37	37	25	5	20
or I + III									
I + II + III	No.	0	2	7	14	9	10	19	12
	%	0	8	30	44	47	63	95	80
None	No.	0	9	3	2	0	0	0	0
	%	0	38	13	6	0	0	0	0
Total number of sera tested		6	24	23	32	19	16	20	15

observed. A serum was considered to possess neutralizing antibodies if one or both tubes showed no cytopathogenic effect. In most cases the results in both tubes were identical.

RESULTS

1. *Antibodies.* Results are summarized in Table 1 and Figure 1. At the age of two years, antibodies to types I and III are already present in 70 and 57 per cent respectively of the sera examined, while antibodies to type II are close to the 50 per cent limit (47%). The percentage of positive sera thereafter rises steadily, reaching 95–100 per cent in the age group 6–7 years. Antibodies to type II show throughout a somewhat slower rise, but they are present in 100 per cent of the sera at the same age as the antibodies to the other types. The early age at which infection with these viruses occurs is brought out clearly by the fact that five of six sera from infants between 6 months and 1 year of age show antibodies to type III, and one of them antibodies to type I. Antibodies against all three types of poliomyelitis virus are present in more than 50 per cent of the sera at the age of four. At school age 95 per cent of the children possess antibodies against all three types.

These results are in agreement with those of a previous survey in Israel (Bernkopf and Levine, 1955) in which 392 sera were examined for Lansing antibodies using white mice as the test animal. It was observed that more than 50 per cent of the sera possessed antibodies at the age of two. Similar results were reported from other countries in the Eastern Mediterranean area. In Cairo (Paul *et al.*, 1952) and French Morocco (Paul and Horstman, 1955) acquisition

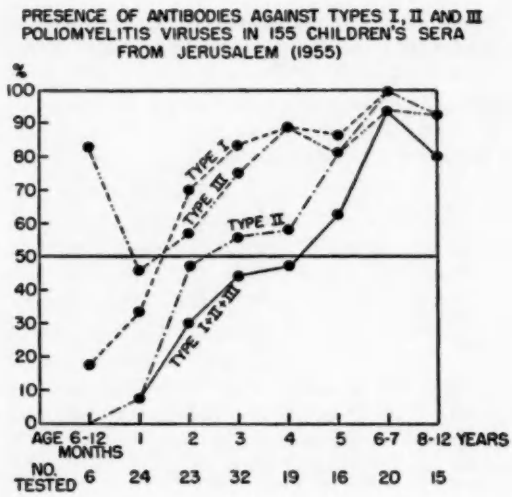

FIG. 1

of antibodies seems to occur somewhat earlier, but the subsequent rise in the percentage of sera containing antibodies resembles the findings in Israel.

Our results fit in with the observation that the majority of cases of paralytic poliomyelitis in this country occur in the age group 0–3 years (Yekutiel *et al.*, 1955), when the percentage of sera containing antibody is relatively low. It is of interest, in this connection, that paralytic disease is caused by strains of all three types, with types I strains prevailing; 30 type I, 8 type II and 4 type III strains were isolated in our laboratory in 1955 while of 70 strains isolated so far in 1956, 67 belonged to type I and 3 to type II.

2. *Socioeconomic factors.* The influence of socioeconomic conditions on the age at which infection first occurs has been frequently stressed (Walton *et al.*, 1955; Melnick *et al.*, 1955). The percentage of children possessing antibodies is found to differ greatly when countries or population groups with widely differing standards of living are compared. It was decided to evaluate the influence of these factors in the community under study, although differences in economic status and living conditions were relatively slight. This is already indicated by the fact that the better-off families did not, in general, have more living space than other families, the few 'large' (three-room) flats being in fact allotted to large and needy families. Generally speaking, it can be stated that even families in the best conditions enjoyed only a moderate standard of living, while in some of the poorer families with many children, near slum conditions prevailed. In the present study three factors are considered: economic status, cleanliness of the home and family size. Evaluation of economic status and cleanliness was based on the intimate knowledge of the family nurse and doctor. No objective criteria were defined for the evaluation of cleanliness; assessments as good, medium or bad represent the family nurse's general impression of the state of cleanliness

of the home, so that it is not possible to determine the relative weight attached to the various elements of home cleanliness. In the case of economic status, the following categories were defined: good (head of the family on full-time work and able to meet the basic needs of the family); medium (head of family on part-time work or, because of many children, only partially able to meet the basic needs of the family), bad (head of family unemployed and family on relief). The third factor, family size, was defined as the total number of persons in the family, including the sample child. The analysis of these factors was limited to children under four years of age, of whom there were 85. They were classified according to the categories described above, as well as according to the number of types for which antibodies were found in the sera (i.e. 0, 1, 2 or 3).

Results are shown in Tables 2, 3 and 4. Frequency of antibodies varied slightly between the three categories of economic status (Table 2), but the differences were not statistically significant. Both cleanliness and family size, on the other hand, were significantly related to antibody frequency: children from homes with a low ranking for cleanliness tended to have more types of antibodies than children from cleaner homes, and antibody frequency increased with family

TABLE 2

Distribution of antibodies according to economic status of family: children aged 6 months–3 years

Antibodies	Economic status							
	Good		Medium		Bad		Total	
	No.	%	No.	%	No.	%	No.	%
None	5	12	6	18	3	25	14	17
One type	18	45	9	27	1	9	28	33
Two types	9	23	7	22	4	33	20	23
Three types	8	20	11	33	4	33	23	27
Total	40	100	33	100	12	100	85	100

$X^2 = 6.82$, n = 6, $P > 0.30$.

TABLE 3

Distribution of antibodies according to cleanliness of home: children aged 6 months–3 years

Antibodies	Cleanliness							
	Good		Medium		Bad		Total	
	No.	%	No.	%	No.	%	No.	%
None	6	21	7	19	1	5	14	17
One type	13	47	9	24	6	30	28	33
Two types	7	25	10	27	3	15	20	23
Three types	2	7	11	30	10	50	23	27
Total	28	100	37	100	20	100	85	100

$X^2 = 13.65$, n = 6, $P < 0.05$.

TABLE 4

Distribution of antibodies according to family size: children aged 6 months–8 years

Number of persons in family	Antibodies				Total
	None	One type	Two types	Three types	
3	2	2			4
4	5	9	6	3	23
5	6	7	3	6	22
6	1	2	4	4	11
7		5	2	5	12
8		3	3	5	11
9			1		1
10			1		1
Total.....	14	28	20	23	85

$X^2 = 10.89$, $n = 4$, $P < 0.05$.

size (Tables 3 and 4). Further statistical analysis, however, revealed a significant negative correlation between cleanliness and family size, suggesting that the correlation between cleanliness and frequency of antibodies might be due to the third factor, family size, and similarly that the operative factor in the relationship between antibody frequency and family size might be cleanliness. Calculation of partial correlation coefficients showed that the elimination of the factor of family size considerably reduced the correlation between cleanliness and antibodies, and the same was true for the role of cleanliness in the association between antibodies and family size, but significant correlations still persisted. In other words, in the present data cleanliness and family size seem independently to influence the frequency of occurrence of antibodies.

SUMMARY

Sera from 155 children in a new housing project near Jerusalem were examined for antibodies against the three types of poliomyelitis. From the age of two to three years onwards more than 50 per cent of the sera showed antibodies against all three types of poliomyelitis. In the age group 6–7 years 95 per cent of the sera contained antibodies against all three types. Antibodies were more frequent in children of families where crowding and a low degree of cleanliness were present. Under the conditions observed no influence of the economical status of the family was apparent.

ACKNOWLEDGEMENT

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NOTE ON ANTIBODIES TO POLIOMYELITIS VIRUSES IN THE SERA OF RESIDENTS OF EASTERN SAUDI ARABIA[1]

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The epidemiology of poliomyelitis has not previously been studied in Saudi Arabia by serologic tests. In neighboring countries, however, the occurrence of antibodies has been reported, for example in Egypt (Paul *et al.*, 1952), Israel (Yekutiel *et al.*, 1955), and Turkey (Payzin, 1956). In connection with a study undertaken for a different purpose, serum specimens were obtained from 57 persons who were life long residents of Eastern Saudi Arabia. This note reports results of tests on those sera for neutralizing antibodies against the 3 types of poliomyelitis virus.

METHODS

The sera were stored in vials at −20°C. Preliminary trials using Salk, Youngner and Ward's metabolism inhibition test (Salk, *et al.*, 1954) showed that in low dilutions the sera were toxic for human liver cells or human embryo fibroblasts. For this reason, subsequent tests were performed with trypsinized human kidney cells. The sera were inactivated at 56°C. for 30 minutes just before use. Dilutions were prepared in isotomic phosphate buffered saline, pH 7.0. Serum dilutions were prepared 18 hours before the addition of virus and kept at +4°C. in stoppered tubes. Sera of adults were tested at dilutions of 1/4 and 1/32. Sera from children were run at 1/4 and 1/16.

Poliomyelitis viruses. Brunhilde (Type I), Lansing (Type II), and Leon (Type III) viruses were supplied through the courtesy of Dr. Franklin A. Neva. Two tenths ml. of virus dilutions, containing 100 $TCID_{50}$ (tissue culture infections dose) of virus, were mixed with 0.2 ml. of serum dilutions. Following one hour's storage at 4°C., 0.1 ml. of the serum-virus mixture was inoculated into each tissue culture tube, using separate pipettes for each serum dilution.

Tissue cultures. Using Rappaport's technic (Rappaport, 1955), cell suspensions were prepared from human kidneys obtained from young hydrocephalic children who had had surgical operations for spinal-ureter anastomosis. According to the number of days of storage of the cell suspensions, 100,000 or 150,000 cells, in a volume of 0.75 ml. medium, were inoculated into each Wasserman tube. The tubes were then stoppered with No. 00 white rubber stoppers and slanted for one week in small aluminum racks at 37°C. On the 7th or 8th day, the medium was replaced with 1.0 ml. fresh medium just before inoculation with the serum-virus mixture. The same medium was used for growing and maintaining the human kidney cells (45% bovine amniotic fluid, 45% Hanks' balanced salt solution, 5%

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TABLE 1

Occurrence of neutralizing antibodies according to age groups and types of poliomyelitis viruses

Age in years	Positive for one type only			Positive for more than one type				Negative	Total
	I	II	III	I + II	I + III	II + III	I + II + III		
<2	0	1	0	0	0	0	1	0	2
2–4	0	0	0	0	1	0	1	1	3
5–9	0	0	0	0	0	0	4	0	4
10–19	0	0	0	0	1	2	0	1	4
20+	4	2	0	4	1	6	13	0	30
ages not known	1	1	0	2	3	0	7	0	14
Totals	5	4	0	6	6	8	26	2	57
%	9	7		10	10	14	46	4	

TABLE 2

Neutralizing antibodies in sera of children

Age in Years	Type I	Titers Against Type II	Type III
18/12	16+*	4	16+
2	0†	16	0
4	16+	16+	16+
4	16+	0	16
4	0	0	0
5	16+	16+	16+
6	16+	16+	16+
6	16+	16+	16+
9	16+	16+	4
10	0	16+	4
10	0	0	0

* 16+ = endpoint not determined
† 0 = < 4

beef embryo extract, 5% inactivated horse serum, sufficient 1.4% bicarbonate solution to adjust to pH 7.4–7.5 and 100 units penicillin and 100 mg. streptomycin/ml.).

Controls. Type specific monkey sera and a low-titer human serum were included in all tests. Virus titrations and normal cell controls, inoculated with phosphate buffered saline, were also included in each test.

RESULTS

Table 1 presents the results of tissue culture neutralization tests according to the antibody content of Types I, II, and III and their combination in specific age groups. Antibodies to one or more types of poliomyelitis virus were detected in 55 of the 57 sera. Twenty-six of the 57 sera were found to have neutralizing

antibodies against all 3 types. The 2 persons whose sera were negative at 1/4 against all 3 types were boys, age 4 and 10 years.

Although the number of sera tested is insufficient for conclusive appraisal, it would seem probable that there is no significant difference between the prevalence of infections of the 3 types of poliomyelitis viruses in this area. Although endpoint titrations were not carried out, it may be noted that, of 42 sera tested in a 1/32 dilution, 28 showed an antibody titer of 1/32 or higher for Type I, 22 for Type II, and 15 for Type III.

The results of the tests on children's sera are shown in Table 2. Although the number is very small, it is evident that poliomyelitis antibodies are common in this sample.

SUMMARY

Fifty-seven blood sera obtained from life-long residents of Eastern Saudi Arabia were tested in tissue culture for neutralizing antibodies against poliomyelitis viruses, Types I, II, and III. Of the 57 sera tested, 55 were positive against one or more types. Antibodies to Type I were detected in 75%, against Type II, 77% and against Type III, 70%. It seems probable that all three types of poliomyelitis are endemic in the area and that exposure occurs at an early age.

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STUDIES ON *PASTEURELLA PESTIS* IN FLEAS

VI. THE LABORATORY CULTURE OF *Xenopsylla vexabilis hawaiiensis* JORDAN, 1932

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Xenopsylla vexabilis hawaiiensis and *Xenopsylla cheopis* are the only two members of Xenopsyllinae known in the Hawaiian Islands. The Hawaiian flea is a distinct subspecies of *X. vexabilis* of Australia (Jordan, 1936; Hopkins and Rothschild, 1953) and is apparently specific for the Hawaiian Islands. In contrast to the predominantly urban habits of the Oriental rat flea, the Hawaiian flea appears to prefer rural field areas and is usually most prevalent on field rodents (Eskey, 1934; Kartman and Lonergan, 1955a). Its role in the maintenance of enzootic plague in Hawaii has been shown by the epidemiological work of Eskey (1934).

In view of the public health importance of *X. v. hawaiiensis*, the Communicable Disease Center undertook experimental work designed to evaluate its efficiency as a host for *Pasteurella pestis* (Kartman *et al.*, 1956). In order to accomplish this, large numbers of fleas were needed and it became imperative that the Hawaiian flea be cultured en masse under laboratory conditions. This report presents methods for, and the results of breeding this flea species, together with notes concerning the flea under natural conditions in Hawaii.

METHODS AND RESULTS

The original specimens of *X. v. hawaiiensis* from which the laboratory culture was established consisted of 4 fleas taken off the native field rat, *Rattus hawaiiensis*, on the Island of Hawaii. These specimens were received via air mail in February, 1954. They were placed on an albino Norway rat which was kept in a glass aquarium filled with sawdust and covered with fine-mesh screen in a room where the temperature fluctuated between 21° to 23°C. and relative humidity varied from 69 to 73 per cent. Powdered beef blood (Difco) was mixed with the sawdust and the rat was fed with pressed food pellets and bits of lettuce and carrot which supplied moisture in place of drinking water. A continuous supply of vegetables undoubtedly increased the humidity within the aquarium.

Within 4 months adult fleas were noted in the aquarium and two new colonies were then initiated in glass aquaria. All three colonies flourished until October, 1954, when the numbers of adult fleas appeared to be decreasing. This may have been due in part to the dry autumn months when it was difficult to achieve a high humidity in the insectary. To increase the humidity, water-soaked sponges were suspended in the aquaria. However, this seemed to favor the development of great numbers of non-parasitic mites (family Acaridae) and the colonies were discarded. Only 11 male and 6 female fleas were recovered at the end of November.

While the aquarium-type breeding chambers were in use, a new type of unit was started in September. These units were similar to those described by Smith and Eddy (1954) and were already being used successfully for the culture of *Xenopsylla cheopis*. The breeding unit consists of an enameled bucket, 31 cm. in diameter and 31 cm. high. The larval substrate is made up of 3 parts of autoclaved white sand to one part of powdered blood and/or other food such as powdered dog-chow pellets. On the sand is placed a copper screen stretched over and attached to a galvanized sheet iron ring one inch wide, made to fit the inside diameter of the bucket. A thin layer of wood shavings is placed on the copper screen to serve as an absorbent material for the host's urine. The host, a young albino Norway rat, is held in a small expanded-metal basket (of the type used to clean laboratory glassware) which rests on the wood shavings.

Adult fleas were placed on the rat and within a period of about 42 days the sand was sifted with a fine mesh strainer. Any pupae present remained in the strainer. These were gathered and placed in beakers for emergence. When the adults emerged they were placed on rats in additional breeding units. After the first sifting, it was found that an interval of about 21 days between siftings provided a maximum yield of pupae. This corresponded with our former results in breeding *X. cheopis*. During a period of 7 days after sifting, the host rat was kept in the breeding unit and was then removed to service other units. Thus, during the remaining 14 days prior to the next sifting, the flea eggs and larvae matured in the host-free unit. This arrangement required the use of two thirds fewer rats and kept the accumulation of urine in the larval substrate to a minimum.

Although the bucket-type breeding units were quite adequate, consistent production of fleas was not attained until better control of humidity was established. With humidistatic control at 93 per cent to 100 per cent relative humidity and with temperature at 23° to 27°C., 4 breeding units yielded up to a maximum of 2,500 pupae. At the time this production started in April, 1955, powdered "Friskie" pellets had successfully replaced the more expensive powdered beef blood. Data on flea production are shown in Table 1. It should be noted that under these conditions *Xenopsylla cheopis* did not breed well and had to be separately maintained at lower atmospheric levels of temperature and humidity. The aquaria breeding units were discarded after being replaced by the bucket-type units. It is of interest to note that fleas in the 4 aquaria units emerged and lived a maximum of 35, 35, 49 and 72 days respectively after removal of the rat.

TABLE 1

Average pupal yield per sifting (every 21 days) of two breeding units for Xenopsylla vexabilis hawaiiensis; *approximately 200 adult fleas were placed on each of two white rat hosts to initiate the colonies in February and March, 1955*

April	May	June	July	Aug.-Sept.*	Oct.	Nov.
650	1100	900	750	—	780	2500

* Production suspended, but colonies maintained by occasional introduction of host.

The only other attempt to breed *X. v. hawaiiensis* was that reported by Eskey (1934) who raised over 500 of these fleas in Honolulu. This author reported that the Hawaiian flea could be bred successfully only when green grass was added to the larval substrate. He used a mixture of rolled barley, dried blood, and grass. The fleas were raised in 5-gallon glass jars and in test tubes.

In the present attempts to breed *X. v. hawaiiensis*, the grass-factor was thoroughly explored. Freshly clipped grass was used both in the aquaria units and in the bucket-type units after improved humidity control had been established, but no differences in flea production were noted. As an example, 2 enameled bucket-units were set up, one with grass plus the usual larval food and the other with wood shavings instead of grass. Within 6 weeks each unit yielded about 500 pupae each. On the basis of our experience, it is suggested that the grass served primarily as a humidifying factor in Eskey's work since, without humidity control, his cultures would tend to have more moisture when the grass was added. The importance of moisture is shown by the fact that our flea colony almost perished at 70 per cent relative humidity, whereas it flourished at 90 per cent or over.

NOTES ON BREEDING IN NATURE

Eskey (1934) suggested that the chief breeding place of *X. v. hawaiiensis* was ". . . probably among vegetation on the surface of the ground". This hypothesis was based upon the fact that he was not able to obtain adult fleas in laboratory cultures unless green grass was used in the larval substrate. During recent work in the Honokaa area, Hamakua coast, Island of Hawaii (Kartman and Lonergan, 1955 a and b) some incidental observations were made which do not support Eskey's hypothesis. Over 60 rat burrows were excavated and about 20 of these yielded nesting materials. Nests were removed together with small amounts of soil underneath them. The burrows were mainly those of the native Hawaiian rat, *Rattus hawaiiensis*. Their burrow systems average 3 to 4 feet in length and 12 to 15 inches in depth. All nests found were built of masses of loosely woven dry grass. No fresh or green grass was ever seen in these nests. In addition to fleas, many of the nests contained mites, *Echinolaelaps echidninus*, *Laelaps nutalli*, *Listrophoroides* sp. on rat hairs, and a species of *Mysolaelaps*. Some rat hairs in the nests had attached louse nits, *Polyplax spinulosa* and *Hoplopleura* sp. Also prevalent were roaches, earwigs, small beetles, spring tails, sow bugs, millipedes, centipedes, and physid snails.

The fleas and mites were removed from nesting material and soil in a Berlese apparatus and the nests were then placed in quart jars and held for flea emergence in a room where the temperature fluctuated between 21° to 23°C. The average annual rainfall in the Hamakua region is approximately 68 inches, thus insuring a relatively high humidity during most of the year. In contrast to this, the annual rainfall in Honolulu where Eskey (1934) raised the *X. v. hawaiiensis* is about 25 inches.

A tabulation of some of the nest findings is presented in Table 2. In the 15 rat nests shown, 217 *X. v. hawaiiensis* were found; of these, 122 or 56.2 per cent

TABLE 2

Xenopsylla vexabilis hawaiiensis *found breeding in rat nests from the Honokaa area, Hamakua Coast, Island of Hawaii.**

Date	Rat nest†	Flea stages found			No. adults emerged	Total adults
		adults	larvae	eggs		
3/25/52	haw.	2	0	0	0	2
3/ 5/53	haw.	7	14	3	10	17
3/ 9/53	haw.	3	8	0	6	9
3/16/53	haw.	9	31	0	24	33
3/17/53	haw.	6	9	0	4	10
3/20/53	haw.	6	8	0	5	11
4/ 1/53	haw.	30	28	0	27	57
4/ 6/53	haw.	2	0	2	1	3
4/ 7/53	haw.	0	4	4	5	5
4/ 8/53	haw.	1	0	0	0	1
4/30/53	norv.	0	18	5	12	12
4/30/53	norv.	8	8	0	5	13
5/ 1/53	alex.	9	20	0	8	17
5/ 5/53	haw.	7	15	0	9	16
5/17/53	haw.	5	8	2	6	11
Totals.....		95	171	16	122	217

* Only nests with fleas are shown; negative nests or those containing other flea species have been omitted.

† haw.—*Rattus hawaiiensis*; norv.—*R. norvegicus*; alex.—*R. rattus alexandrinus*.

emerged after collection of the nests. The adults which emerged represented a maturation of about 65.2 per cent of the 187 larvae and eggs noted in the nesting material. These data suggest that *Xenopsylla vexabilis hawaiiensis* breeds in rat nests and does not require green grass as a larval food. This appears to explain our success in laboratory cultivation of the Hawaiian flea under conditions of high humidity, but with the usual larval food used in culturing *X. cheopis*.

SUMMARY

Large numbers of *Xenopsylla vexabilis hawaiiensis* were needed to carry out tests of its efficiency as a plague vector. Efficient culture of the Hawaiian flea was accomplished in white enameled buckets containing a housed host rat together with a larval substrate consisting of sand and powdered dog pellets (Friskies). The pupae were kept at a temperature of from 23° to 27°C. and a relative humidity of from 93 per cent to 100 per cent. These conditions differed from those required by *Xenopsylla cheopis*, which was maintained separately at lower temperature and humidity. The pupae were sifted from the sand approximately every 3 weeks for maximum production.

In nature, *X. v. hawaiiensis* was found to breed in rat nests which consisted mainly of dried grass leaves. This evidence, together with the results of laboratory culture, suggests that the fleas probably do not breed on the surface of the ground, nor do the larvae require green grass as formerly postulated.

ACKNOWLEDGMENTS

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A SURVEY OF *SCHISTOSOMA HAEMATOBIUM* INFECTIONS IN MAN IN LIBERIA

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Previous workers (Vogel, 1932; Harley, 1933; Veatch, 1946) have reported the occurrence of schistosomiasis in man in Liberia, but none has provided data on the distribution of these infections throughout the country. The present report describes the results of a country-wide survey of *S. haematobium* infections.

MATERIALS AND METHODS

Adult male employees (mostly young) of two rubber plantations and a mining company were studied.[1] Most of the subjects were examined at the Harbel Plantation of the Firestone Plantations Company where some 20,000 men are employed. They originate in widely scattered regions throughout the country, the majority coming directly from their native villages to the Plantation area. An intensive search of this area by Dr. Elmer Berry (personal communication, 1950) failed to reveal the presence of snail hosts of the schistosome parasites of man and indicated the absence of transmission. To provide additional information for the Western Province and the coastal half of the Eastern Province further subjects were studied at the Cavalla River Plantation of the Firestone Plantations Company situated near Cape Palmas, the Liberia Mining Company at Bomi Hills and the B. F. Goodrich Plantation near Bomi Hills. Available information indicates that these areas are also free of the snail hosts of schistosome parasites of man and that transmission is absent.

Urine specimens were examined from 3,429 individuals representing most regions of the country. They made up some 40 regional groups and in about half of these at least 100 persons were examined; in the remaining groups smaller numbers were seen, sometimes as few as 10 persons.

Diagnosis was based on the examination of a single urine specimen collected in the late morning or early afternoon, after the subjects had been exercising. At the same time information was obtained on the original place of residence of each subject and, in certain instances, on all other areas where he had lived. Nearly all knew the names of their Paramount Chiefs which made it easy to identify their place of origin.

Urine specimens were collected in urinalysis bottles and allowed to settle; the supernate was then poured off leaving enough to fill a 15 ml. centrifuge tube. This was centrifuged, the liquid supernate decanted and the entire sediment transferred to a glass slide and examined for the presence of schistosome eggs. All urine specimens were examined by the writer.

[1] The co-operation of the Firestone Plantations Company, The Liberian Mining Company, and The B. F. Goodrich Company in this survey is acknowledged with thanks.

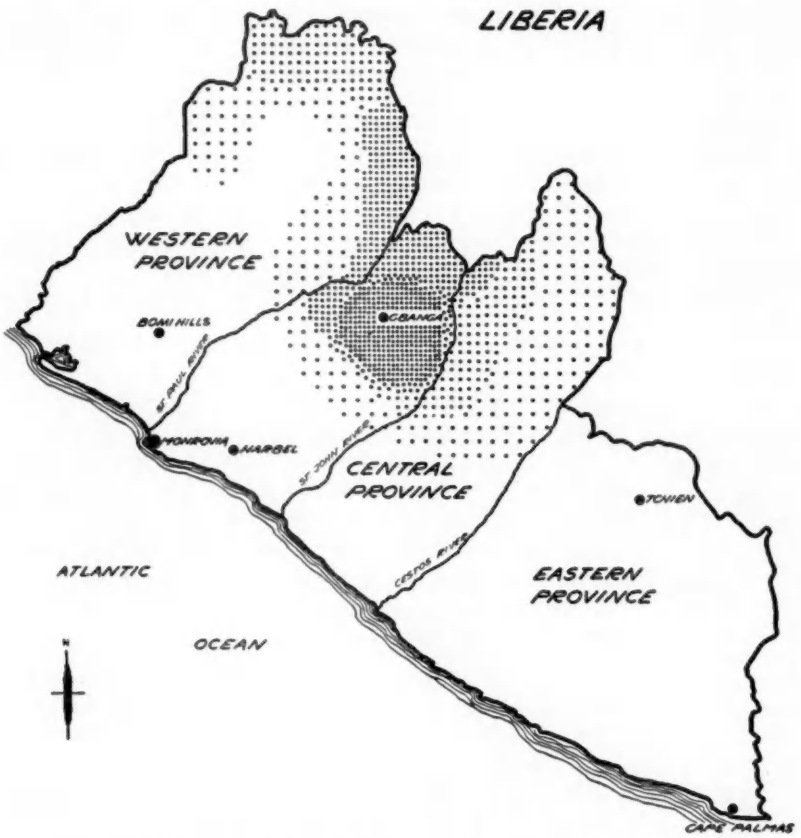

FIG. 1. Distribution of *Schistosoma haematobium* infections in Liberia

RESULTS

Of the 3,429 individuals examined 865 or 26 per cent were found positive for *S. haematobium* eggs. However, this figure has little significance as the incidence of infection was found to be determined by the numbers of individuals coming from areas of high endemicity. A study of the incidence in the different regional groups revealed that large parts of the country were apparently free from infection, while in others high infection rates prevailed. The distribution and comparative degrees of endemicity are shown in Figure 1. All shaded areas in the map indicate the place of origin of regional groups who showed infection rates of at least 17 per cent. The more densely shaded areas represent progressively higher infection rates, the highest rate found being 67 per cent. While groups from the unshaded areas were, for the most part, free from infection, occasional individuals in the lower half of the Central Province were found to be passing

eggs of *S. haematobium*, although the infection rate from this region never exceeded 10 per cent. In a few cases it was possible to determine that these infected individuals had visited known endemic areas and it was concluded that all such infections were acquired elsewhere. More detailed surveys may reveal, however, that endemic pockets of infection do occur in the region.

The inability to get accurate information from every individual studied, and the small numbers available for examination from certain regions, make the results of limited value as a precise quantitative index of *S. haematobium* infection for all localities of the country. Nevertheless, it is believed that the general distribution of this infection in Liberia has been established.

Eggs of *S. mansoni* were seen in approximately 0.3 per cent of all urine specimens.

SUMMARY

By examining the labor populations, recruited from different parts of the country, of several rubber plantations and a mining company located in areas where transmission of *Schistosoma haematobium* has never been known to occur, it has been possible to determine the general distribution and comparative intensity of this infection throughout Liberia. The coastal half of the country was found to be free of infection as was the entire Eastern Province and much of the Western Province. The highest infection rates were centered in the upper part of the Central Province between the St. Paul and St. John rivers; groups from this region showed infection rates above 50 per cent.

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DISTRIBUTION AND PREVALENCE OF HUMAN SCHISTOSOMIASIS IN PUERTO RICO IN 1953[1]

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In Puerto Rico schistosomiasis has been known since 1904, when Dr. I. González-Martinez established its presence (Hoffman, 1927). Since that time numerous investigators have studied the disease (see Hoffman and Faust, 1934; and Weller and Dammin, 1945, for literature review).

The purpose of this study was to obtain epidemiological information on the present incidence and distribution of *Schistosoma mansoni* infections as a basis for the ultimate control of schistosomiasis in Puerto Rico.

In the overall study three aspects were considered: the incidence and geographic distribution of human infections of schistosomiasis; the abundance and distribution of *Australorbis glabratus*, the snail intermediate host of *S. mansoni*; and the relation of human *S. mansoni* infections to similar infections in *A. glabratus*. This paper is concerned only with the first phase.

INCIDENCE AND GEOGRAPHIC DISTRIBUTION OF *SCHISTOSOMA MANSONI* INFECTIONS IN THE HUMAN POPULATION

The municipalities selected for the survey were located in different physiographic and climatic regions of Puerto Rico. (Tables 1 and 2 and Figure 1). Pupils in the first 12 grades (5 to 18 years old) were surveyed with the exception of the school in Rio Piedras where children 1 to 16 years old were sampled. School children were selected because they were easy to sample and represented a relatively uniform group in which sampling design could be regulated. This group of subjects also allowed opportunity for further study to gain insight into changes that might occur in the incidence of schistosomiasis, since a change in schistosomiasis level is likely to occur earlier in this age group than in older individuals.

Before collecting samples in an area, the nature of the program was explained to the municipality's superintendent of schools. A list of all the schools in the area with the grades and number of pupils in each was obtained from the superintendent. This information made it possible to select schools which were representative of the municipality. The superintendent notified each school of intended visits.

The day before samples were taken, half-pint ice cream containers were distrib-

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TABLE 1

The incidence of human schistosomiasis in municipalities in various physiographic and climatic regions in Puerto Rico[a]

Region (See Figure 1)	Municipality	Present Study		Weller and Dammin	
		Number examined	Per cent infected	Number examined	Per cent infected
Y	Jayuya	327[b]	23.2	81	37.0
Q	Rincon	632	0.8	51	0.0
C	Ceiba	273[b]	29.3	160	20.6
A	Quebradillas	634	0.0	108	2.6
B	Vega Baja	935	0.0	208	0.5
	Rio Piedras	199[c]	24.1	1,050	16.5
	Carolina	944	4.4	166	1.2
		2,078	4.3	1,424	12.4
C	Patillas	1,542	27.4	128	20.3
	Arroyo	216[d]	16.7	121	8.3
	Guayama	748[d]	20.1	393	29.0
		2,506	24.3	642	23.4
N	San German	1,144	0.6	354	3.4
O	Anasco	548	2.9	102	25.5
H	Ponce	1,270	0.3	1,230	4.1
V	Caguas	521	29.9	549	25.9
	San Lorenzo	622	8.4	162	11.1
	Guarbo	597	3.2	59	30.5
	Juncos	539	10.0	119	18.5
		2,279	12.3	889	22.5
Grand total and average		11,690	10.0	5,041	13.5

[a] The results of Weller and Dammin (1945) include surveys of young adult males (18–37 years) and those of the present study include surveys of school children (1–18 years).

[b] Dr. Jose F. Maldonado, Professor of Parasitology, School of Tropical Medicine, University of Puerto Rico, conducting a survey of the human population in Puerto Rico for a different study on a U. S. Army grant, kindly supplied the data on the incidence of schistosomiasis in school children for the municipalities of Ceiba and Jayuya.

[c] Survey of children 1–16 years in communities associated with the Quebrada Sabana Llana and Dona Ana in Rio Piedras.

[d] Survey data include only first grade children.

TABLE 2

The distribution of Australorbis glabratus *and* Schistosoma mansoni *populations in various physiographic regions, climatic regions, and aquatic environments in Puerto Rico*

Physiography	Climate	Aquatic environment	Regions[a]
Volcanic and sedimentary rock, Cretaceous, inland, large deposit	Humid, rainfall constant,[b] 60–80 inches per yr.	Many rivers, streams, and ponds,—few reservoirs	F, T, X, Y
	Humid, rainfall sporadic,[c] 60–80 inches per yr.	Many rivers, streams, and ponds	Q
	Subhumid, rainfall constant,[b] 45–60 inches per yr.	Many rivers, streams, and ponds,—few reservoirs	M
Volcanic rock, Cretaceous, coastal, large deposit	Arid, rainfall constant,[b] 30–45 inches per yr.	No permanent water bodies	K
Volcanic rock, Cretaceous, inland, large deposit	Rainy, mountain, rainfall constant,[b] 175 inches per yr.	Many rivers, streams, and ponds	U
Sedimentary rock, Cretaceous, coastal, large deposit	Humid, rainfall constant,[b] 60–80 inches per yr.	Many rivers, streams, and ponds—few lagoons and marshes	C
Limestone, Tertiary, coastal, large deposit	Subhumid, rainfall constant,[b] 45–60 inches per yr.	Few rivers, irrigation canals, limestone sink holes	A
	Arid, rainfall constant,[b] 30–45 inches per yr.	No permanent water bodies	J
Limestone, Tertiary, inland, large deposit	Humid, rainfall constant,[b] 60–80 inches per yr.	Few rivers, streams, limestone sink holes, reservoirs	S
Alluvium, coastal, large deposit	Humid, rainfall constant,[b] 60–80 inches per yr.	Many rivers, streams, ponds, drainage canals, —few lakes, lagoons	B, D, G
	Humid, rainfall sporadic,[c] 60–80 inches per year	Many rivers, streams, ponds, drainage canals,—few lagoons, marshes	N, O, R
	Arid, rainfall constant,[b] 30–45 inches per yr.	Many irrigation canals and impoundments—few rivers, streams, lagoons	H, I
Alluvium, coastal, small deposit	Humid, rainfall constant,[b] 60–80 inches per yr.	Few rivers and streams	E
	Humid, rainfall sporadic,[c] 60–80 inches per yr.	Few streams	P

TABLE 2—*Continued*

Physiography	Climate	Aquatic environment	Regions
Alluvium, inland, large deposit	Humid, rainfall constant,[b] 60–80 inches per yr.	Many rivers, streams, ponds	V, W
	Subhumid, rainfall constant,[b] 45–60 inches per yr.	Many drainage canals, lakes, marshes—few streams	L
Alluvium, inland, small deposit	Humid, rainfall constant,[b] 60–80 inches per yr.	Many rivers, streams, and ponds—few reservoirs	Scattered through sections T, X, Y
	Subhumid, rainfall constant,[b] 45–60 inches per yr.	Many rivers, streams, and ponds—few reservoirs	Scattered through M

[a] See Figure 1 for regions.
[b] Little seasonal variation in rainfall.
[c] Great seasonal variation in rainfall.

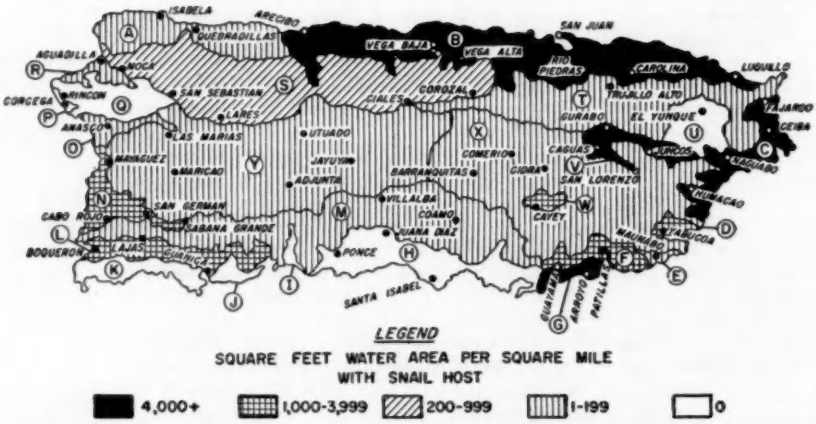

FIG. 1. Distribution of *Australorbis glabratus* in different physiographic and climatic regions in Puerto Rico. [Regions after Myerhoff (1933) and Pico (1950).]

uted to the students of the school. Each container was given a number which corresponded with the number assigned to each student. A record of the pupil's sex, age, and area of habitation (rural or urban) was kept. The pupils were impressed with the importance of providing a fecal sample, and on the following day the boxes were collected and returned to the U. S. Public Health Service Laboratory in San Juan where they were refrigerated until examined. Usually one collection of samples was made each week. Schools were used for collection centers in all areas except Rio Piedras and Ponce where house to house collections were made and samples taken from similar age groups.

TABLE 3

A comparison of Schistosoma mansoni *infection rates in males and females 5 to 18 years old in various municipalities in Puerto Rico, 1953*

Municipality	Number examined		Per cent infected	
	Male	Female	Male	Female
Jayuya[a]	187	140	24.1	22.1
Rincon	341	291	0.3	1.4
Ceiba[a]	139	134	33.8	24.6
Carolina	472	472	7.2	1.7
Patillas	782	759	32.5	22.1
San German	629	515	1.1	0.0
Anasco	292	256	4.5	1.2
Ponce	620	650	0.7	0.0
Caguas	267	251	35.2	23.9
San Lorenzo	337	285	9.2	7.4
Gurabo	320	277	4.4	1.8
Juncos	278	261	14.7	5.0
Totals and averages	4,664	4,291	12.5[b]	8.1[b]

[a] Survey data kindly supplied by Dr. Jose F. Maldonado, Table 1.

[b] Difference between male and female infections significant at odds greater than 99:1.

The sodium sulfate-triton-ether technique (Maldonado, 1953, from Faust and Ingalls, 1946) was employed in examining fecal samples. The procedure was as follows: one gram of feces was placed in a test tube with 5 ml. of sodium sulfate (specific gravity 1.08) and 1 drop of Triton X-100;[5] the mixture was macerated and strained through gauze to remove solid material. The fluid was placed into a 15 ml. centrifuge tube and an equivalent amount of ether was added. This mixture was shaken, centrifuged at 2,000 rpm for 1 minute, and the liquid was then decanted. Two drops of saline were added to the residue and the contents were pipetted onto a slide for microscopic examination. In addition to *S. mansoni*, other helminths observed in the sample were recorded. The accuracy of this method was shown by the fact that 78.5 per cent of the positives in a normal population were detected on an initial examination (Table 7).

The incidence of schistosome infection in all the regions surveyed ranged from 0 to 29.3 per cent (Table 1) with an average of 10.0 per cent. In isolated communities like the one surveyed at the Rio Bairo, as high as 78.6 per cent of the children were found positive (Table 7). With few exceptions, the present results were comparable to those of Weller and Dammin (1945) even though the surveys were made at different times and with different age and sex groups (Table 1).

The males had significantly higher infection rates than the females at odds greater than 99:1 (Table 3). It seems quite plausible that boys are more attracted than girls to water for playing and swimming, are used more in hauling water,

[5] Product of Rohm and Haas Company, Philadelphia, Pa. Trade names are used for identification purposes only and their use does not constitute endorsement by the Public Health Service.

TABLE 4

Incidence of Schistosoma mansoni *infection in school children of various ages in 12 Puerto Rican communities, 1953*

Age	Number examined			Per cent infected		
	Total	Male	Female	Total	Male[a]	Female[b]
5	9	4	5	0.0	0.0	0.0
6	690	320	370	3.2	3.1	3.2
7	1,055	522	533	7.7	8.8	6.6
8	1,144	576	568	8.0	8.7	7.2
9	1,156	583	573	6.3	7.5	5.1
10	1,378	683	695	7.5	8.2	6.9
11	1,220	632	588	6.9	8.2	5.4
12	1,288	676	612	12.6	16.3	8.5
13	1,006	550	456	12.0	13.1	10.7
14	725	421	304	12.0	15.4	7.2
15	462	280	182	12.3	14.6	8.8
16	259	156	103	13.9	19.9	4.9
17	68	46	22	14.7	13.0	18.2
18	21	17	4	9.5	11.8	0.0

[a] The correlation between age and infection rate in the males is significant at odds greater than 99:1 ($r = 0.89$).

[b] The correlation between age and infection rate in the females is not significant ($r = 0.38$).

and are thus more frequently exposed to infection with *S. mansoni*. The relative infection rates of females may increase when they become adults and assume such household chores as washing clothes (Hoffman and Faust, 1934).

The males' ages were highly correlated ($r = 0.89$, Table 4). Hoffman and Faust (1934) found that school children at Barranquitas began swimming in a particular bathing site "about the time they were in the fifth-grade age group." Their observations agree favorably with the present findings since it is assumed that they were speaking about boys, for the girls seldom swim in the streams. Thus the higher rate of infection in the older boys was probably due to increased swimming activity as they became older. The presence of infections in the younger boys and in girls of most age groups, and some of the infection in the older boys, were probably due to activities other than swimming, such as drinking, bathing, fording streams, hauling drinking water, and watering stock in infected streams.

The difference between the infection rates in rural (8.1 per cent) and urban dwellers (12.2 per cent) was not significant (Table 5). Approximately twice as many children were examined from rural areas (5,916) as from urban areas (2,443).

High infection rates with other helminths (hookworms *Necator and Ancylostoma*, 17.1 per cent; *Ascaris lumbricoides*, 20.3 per cent; and *Trichuris trichiura* 92.6 per cent) also occurred in the population sampled (Table 6). Multiple infections with these parasites along with *S. mansoni* weaken the host more than the *S. mansoni* infection alone. Anything which can be done to elimi-

TABLE 5

A comparison of Schistosoma mansoni *infections in school children in rural and urban areas of various municipalities in Puerto Rico, 1953*

Municipality	Number examined		Per cent infected	
	Rural	Urban	Rural	Urban
Rincon	388	244	0.0	2.0
Carolina	554	390	6.3	1.8
Patillas	1,255	287	21.7	52.3
San German	752	392	0.4	1.0
Anasco	310	238	1.0	5.5
Ponce	956	314	0.4	0.0
Caguas	261	260	34.5	25.4
San Lorenzo	502	120	1.8	35.8
Gurabo	509	88	3.5	1.1
Juncos	429	110	10.3	9.1
Totals and averages	5,916	2,443	8.1[a]	12.2[a]

[a] Difference between infections in rural and urban dwellers is not significant (5 per cent level).

TABLE 6

The incidence of hookworms (Necator *and* Ancylostoma), Stronglyoides stercoralis, Ascaris lumbricoides, *and* Trichuris trichiura *in school children from various municipalities in Puerto Rico, 1953*

Based on use of sodium sulfate, triton, ether centrifugation method of stool testing

Municipality	Number examined			Per cent infected with helminth parasites					
	Total	Male	Female	Hookworms			*Strongyloides stercoralis*	*Ascaris lumbricoides*	*Trichuris trichiura*
				Total	Male	Female	Total	Total	Total
Rincon	632	341	291	8.4	10.3	6.2	0.0	10.1	93.5
Ceiba[a]	273	139	134	16.8	19.4	14.2	0.0	5.1	70.3
Quebradillas	634	336	298	12.1	14.9	9.1	0.0	17.2	95.1
Vega Baja	935	486	449	11.6	13.2	9.8	0.3	20.3	94.4
Carolina	944	472	472	11.0	11.4	10.6	0.0	12.9	96.4
Patillas	1,542	782	759	38.8	42.3	35.3	0.0	34.8	97.2
San German	1,144	629	515	5.9	5.9	5.8	2.2	12.7	86.7
Anasco	548	292	256	21.0	20.2	21.9	2.6	21.9	92.3
Ponce	1,270	620	650	2.9	4.5	1.4	0.2	33.8	92.2
Caguas	521	267	251	11.3	13.9	8.8	0.0	17.1	91.7
San Lorenzo	622	337	285	25.9	29.7	21.4	0.2	27.3	93.4
Gurabo	597	320	277	37.7	43.4	31.0	0.0	9.5	92.0
Juncos	539	278	261	17.4	22.3	12.2	0.0	5.6	91.1
Totals and averages	10,201[b]	5,299	4,898	17.1[b]	19.3[c]	14.7[c]	0.4[d]	20.3[d]	92.6[d]

[a] Survey data kindly supplied by Dr. Jose F. Maldonado (Table 1).

[b] Includes four with sex not recorded.

[c] Difference between male and female infections is significant at odds greater than 99:1.

[d] Difference between male and female infections was not significant (5 per cent level).

nate these additional parasites would greatly aid the health and welfare of people in the *S. mansoni*-infected areas.

RELATIONSHIP OF THE ABUNDANCE AND GEOGRAPHIC DISTRIBUTION OF *AUSTRALORBIS GLABRATUS* AND ITS INFECTIONS, TO MAN AND HIS INFECTIONS

To complete the picture of the relationship between the abundance and distribution of snails and their *S. mansoni* infections, and the incidence of human schistosomiasis in Puerto Rico, data from a study on the geographic distribution of *A. glabratus*, with notes on *S. mansoni* infections (Pimentel and White, ms.), will be briefly mentioned.

In general, the incidence of human schistosomiasis was directly related to the abundance and distribution of *A. glabratus*. Only two areas provided exceptions to this relationship. In region "N", a large marsh had an abundance of snails but the human infection was low (Table 1, Figure 1, and Table 2). The other exception occurred in region "Y", a small alluvial valley in the mountains where human schistosomiasis was high and snail abundance was low (Table 1, Figure 1, and Table 2). In these small mountain alluvial valleys snails are scarce most of the time due to the easy flushing of the streams. However, it is quite possible during years of low rainfall for large snail populations to exist. People living in these narrow valleys have close contact with the adjacent water bodies, thus affording excellent opportunity for infection of the snails and of human exposure to *S. mansoni* (Pimentel and White, in ms.). The *S. mansoni* infections in man are detectable after the snails have disappeared from the water body for at least 5 or 10 years since they can be maintained in man for 20 years or more (Manson-Bahr, 1941).

In an effort to relate *S. mansoni* infection rates in man to those in snails, 5,185 snails were examined during April and May, 1953. These snails were collected from 116 well-distributed stations in Patillas municipality. The snails were held for three days in the laboratory and then examined for the liberation of *S. mansoni* cercariae. Only four infected snails were found among those collected and these were from the Quebrada Mamey in the town of Patillas. This infection rate of only 0.08 per cent for the 5,185 snails examined was surprisingly low since 27.4 per cent of the human population surveyed were infected with *S. mansoni* (Table 1).

As a result of the Patillas snail infection survey a more intensive quantitative study in communities associated with a tributary of the Quebrada Sabana Llana, the Quebrada Dona Ana and the Rio Bairoa was initiated. In all these communities a thorough examination for *S. mansoni* was made of 288 children, ranging in age from 1 to 16 years. After the first survey, a repeat survey was made 3 weeks later of all children found negative on the initial survey. A second repeat survey was made on children negative after the first repeat survey.

At the Rio Bairoa community, 78.6 per cent of the children were found to be infected with *S. mansoni*, while at the Quebrada Sabana Llana tributary and Quebrada Dona Ana 27.0 and 35.2 per cent respectively were positive (Table 7).

TABLE 7

Incidence of human schistosomiasis in identified children, aged 1 through 16 years, in three study communities, 1953

Community	Number examined	Per cent infected at completion of		
		Initial survey	Repeat survey No. 1[a]	Repeat survey No. 2[b]
Quebrada Sabana Llana Tributary	74	21.6	24.3	27.0
Quebrada Dona Ana	125	24.0	32.7	35.2
Rio Bairoa	89	66.3	76.4	78.6
Total and averages	288	36.5	44.1	46.5
Per cent of total number positives found (cumulative)		78.5	94.8	100.0

[a] Repeat survey run only on all negatives found on initial survey.

[b] Repeat survey run only on all negatives found on repeat survey No. 1.

Children as young as 2 years of age were found infected. These findings substantiate the early work of Hoffman and Faust (1934). It is interesting to note that 78.5 per cent of the total number of positives, detected in the three surveys made of the stream and river communities, were found on the initial survey (Table 7). The fact that such a large percentage of the total positives was detected on the initial survey gives added confidence in the fecal examination technique utilized.

The snail population measured at 20 permanent stations, 5 in each of 4 well distributed areas along the length of each water body associated with a particular community, varied little during the 6-month study period. When possible, 100 *A. glabratus* were collected monthly from each of the four areas in each of the water bodies. The infection rates of the snails were measured by holding and checking the snails for cercarial liberation for 3 days. After this time they were returned to the location in each stream in which they had been collected. Generally, the *S. mansoni* infections in the snails were very low (Table 8). It was not until the third monthly survey and after a total of 1,352 snails had been examined that a few infected snails were detected at the Rio Bairoa community where 78.6 per cent of the children were infected (Tables 7 and 8). Thus these findings substantiate the results obtained in the previously mentioned study at Patillas.

The infection rates in the snail population varied from month to month (Table 8). Part of this may be due to the sampling technique or because too few snails were examined. But infection in the snail population certainly varies in time more rapidly than infection in man. Infected *A. glabratus* presumably pass cercariae as long as they remain alive. (Faust and Hoffman, 1934). The average length of life of *A. glabratus* is one year (Pimentel, in press) but with light infections of *S. mansoni*, *A. glabratus* survived for only 6 months, and with heavy infections the snails died in a shorter time (Faust and Hoffman, 1934). It is also known that infected snails are more apt to be killed by marked changes in the environment

TABLE 8

Infection rates of Australorbis glabratus *with* Schistosoma mansoni *in three study communities, 1953*

Month	Water body					
	Sabana Llana Tributary		Dona Ana		Bairoa	
	Number snails	Per cent infected	Number snails	Per cent infected	Number snails	Per cent infected
January	58	0	169	0.6	339	0
February	12	0	138	1.4	400	0
March	60	8.3	180	8.3	583	1.7
April	207	0	162	0	414	0
May	207	0	104	0	450	0
June	107	0	186	0	247	0

than are healthy snails (Brumpt, 1941). Man on the other hand can retain infection for over 20 years (Manson-Bahr, 1941).

The Quebrada Sabana Llana, Quebrada Dora Ana, and Rio Bairoa communities are located on alluvium. The level land adjoining the water bodies allows the houses and activities (defecation included) to be located away from the water. Thus, even though snails are continuously present, the infection rate in them is low since only a small amount of feces gets into the streams. The people in this habitat get their infections in small but more or less continuous dosages. A contrasting situation exists in the small mountain alluvial valley at Comerio. The houses are crowded close to the Rio de la Plata and most of the sewage gets into the river. When snails abound during low rainfall periods they are highly infected. A period of low rainfall like that experienced in 1952 may occur once in 5 or 10 years. However, most of the human population could become infected during one of these low rainfall periods, because with such highly infective water it would take only one short exposure to become heavily infected with *S. mansoni*.

When school is in session, many of the school children at Comerio are exposed to the Rio de la Plata water, since one of the largest schools is located across the river from the main section of the town. Instead of taking the roadway which is about 1 mile farther and partly uphill, most of the children cross the river by means of rocks, automobile tires, and boards. Many of the children, if they are wearing shoes, take them off so as to keep them dry as they cross the so-called bridge. This practice affords an ideal way of infecting the community any time ecological conditions are suitable for *A. glabratus* and *S. mansoni*.

SUMMARY

During 1953 a single fecal sample from each of over 10,000 Puerto Rican school children was examined for helminth eggs, especially those of *S. mansoni*. The following average percentage rates of infection were found: *S. mansoni*, 10.0; hookworm (*Necator* and *Ancylostoma*), 17.1; *Trichuris trichiura*, 92.6; *Ascaris lumbricoides*, 20.3; and *Strongyloides stercoralis*, 0.4. The incidence of *S. mansoni* in boys and girls was 12.5 and 8.1 per cent, respectively. This difference was signifi-

cant at odds greater than 99:1. A high degree of correlation (r = 0.89) was found between the age of the boys and their rate of *S. mansoni* infection, while with the girls, these factors were not correlated (r = 0.38).

In general, the abundance of the snail vector (*Australorbis glabratus*) was related directly to the incidence of human schistosomiasis. There was little or no agreement between *S. mansoni* infection rates in snails and those in human beings at a given time in a particular locality. The epidemiological aspects of these findings are discussed in the light of results obtained from a study of human beings, snails and *S. mansoni* infections in three stream communities and from other studies in selected regions in Puerto Rico.

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SERODIAGNOSIS OF SCHISTOSOMIASIS: SPECIFICITY OF THE COMPLEMENT-FIXATION TEST IN OTHER HELMINTHIC INFECTIONS

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Chaffee *et al.* (1954) demonstrated that extraction of adult *Schistosoma mansoni* with ether prior to extraction with buffered saline removed substances which fixed complement with syphilitic serum; the result was a significant improvement in specificity of the diagnostic test without an appreciable loss of reactivity with serum from schistosome infections. Sera from 96 of 98 persons infected with *S. mansoni* reacted with the antigen, while sera from 46 healthy persons, 28 syphilitic persons, and four persons with hookworm infection gave no reaction. In a collateral survey among 276 Puerto Rican soldiers, using the same purified antigen, serologic reactions occurred in 43.5 per cent of the group, although in only 18.8 per cent were eggs of *S. mansoni* found in the stools (Horstman *et al.*, 1954). The present study was undertaken to elucidate the possible effect of other helminth infections upon the specificity of the complement-fixation test.

MATERIALS AND METHODS

Sera. Sera representing single and multiple helminth infections were obtained from 69 Puerto Ricans, 39 Trinidadians, and 8 Japanese. The sera from the 8 Japanese, of Shizuoka prefecture, were from acute and chronic infections of *Paragonimus westermani*. Bloods were placed in the refrigerator (3–6°C.) from 4 to 6 hours after collection, and the serum was removed within 48 hours. All sera were held at −20°C. until tested, at which time they were thawed and the required amount heated at 56°C. for 30 minutes.

Stools. The presence of parasites in Puerto Ricans was determined by multiple stool examinations using the AMS III concentration technique (Hunter *et al.*, 1948) and alcohol sedimentation (Jahnes and Hodges, 1947). For the purpose of this study, Puerto Ricans with 3 negative stools were considered free of schistosomiasis. A single stool specimen was obtained from each Trinidadian, approximately 1 gram of which was well emulsified in 10 cc. of MIF solution (Sapero and Lawless, 1953) and refrigerated at 3–6°C. until examined by direct smear and by AMS III concentration technique.

Complement-fixation procedure. The technique of the complement-fixation test and the preparation of the antigen were performed as previously described (Chaffee *et al.*, 1954). Aliquots of 0.3 ml. volume were allowed for each of the five required reagents. To 0.1 ml. of undiluted, heated serum plus 0.2 ml. of VBS[2] was added 0.3 ml. of complement ($3C'_{H50}$) and 0.3 ml. of antigen (a stand-

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[2] The stock solution consisted of 83.8 gm. NaCl, 2.52 gm. $NaHCO_3$, 3.00 gm. Sodium

TABLE 1

Helminth distribution in 108 parasitized individuals

Source	No. in group	No. of individuals with				No. of individuals with		
		Tt.*	Hwo.†	Al.‡	Ss.§	3 helminth Spp.	2 helminth Spp.	1 helminth Sp.
Puerto Ricans	69	64	44	20	2	13	35	21
Trinidadians	39	17	17	15	1	1	9	29
Totals	108	81	61	35	3	14	44	50

* Tt = *Trichuris trichiura*
† Hwo = Hookworm
‡ Al. = *Ascaris lumbricoides*
§ Ss. = *Strongyloides stercoralis*

ardized, buffered saline extract of ether-extracted adult *S. mansoni*[3]). Serum, complement, antigen, and cell controls were adjusted to standard volume by the addition of VBS. After incubation of test and control tubes for 16 to 18 hours at 3–6°C., 0.6 ml. of optimally sensitized sheep cells (Kent, 1947) were added to all tubes, which were then placed in the 37°C. water bath for 30 minutes. The tubes, after centrifugation, were then visually compared with standards simulating various degrees of hemolysis; and the results were recorded as 4+, 3+, 2+ (reactive); 1+, ±, (weakly reactive); and − (non-reactive).

RESULTS

Trichuris trichiura and hookworm proved to be the most common parasites in both the Puerto Ricans and the Trinidadians, although *Ascaris lumbricoides* and *Strongyloides stercoralis* were also found. Over half the sera tested were from individuals harboring two or more parasites (Table 1). Sera from none of the 39 Trinidad natives and from only two of the 69 Puerto Ricans reacted in the complement-fixation test for schistosomiasis. Examination of three stools from one, a 42-year-old man raised in Bayamon, Puerto Rico, failed to demonstrate eggs of *S. mansoni*. Examination of three stools and a rectal biopsy from the other, a four-year-old boy who lived near Arecibo, failed to demonstrate *S. mansoni* infection. A differential blood film showed 60 per cent eosinophiles and the clinician's provisional diagnosis on the basis of clinical findings was acute schistosomiasis.

The sera from 2 of the 8 Japanese infected with *P. westermani* also reacted in the test for schistosomiasis. One patient, whose serum was weakly-reactive (±),

5,5-diethyl barbiturate, and 4.60 gm. 5,5-diethyl barbituric acid in distilled water sufficient to make 2,000 ml. (Mayer *et al.*, 1948). The acid was dissolved in 500 ml. hot distilled water and added to the solution of the other reagents. The whole was then cooled to room temperature, and made up to 2,000 ml. with distilled water. Before use, one volume of the stock solution was diluted by adding four volumes of distilled water; the pH of the diluted solution was 7.3–7.4.

[3] Adult *S. mansoni* were harvested from white mice six to eight weeks after exposure by tail immersion.

had a recent paragonimiasis (3 months duration); in the other, whose serum was reactive (2+), the infection was of two years duration. Eggs of the parasite were demonstrable in the sputum of both at the time of blood collection. Skin tests for schistosomiasis were negative.

DISCUSSION

The nematode parasites of the two sero-reactive Puerto Ricans did not differ from those of the other 67 Puerto Ricans and 39 Trinidad natives who were non-reactive serologically. Although eggs of *S. mansoni* were not demonstrable in either, the possibility of schistosome infection cannot be excluded since both individuals lived in areas where schistosomiasis has been endemic (Weller and Dammin, 1945). Persistent examination of excreta might have revealed eggs of the parasite. Baroody and Most (1946) found that in some light infections with *S. japonicum* it was necessary to examine 20 to 30 stools to establish the infection. The high eosinophilia (60%) and clinical findings of the four-year-old boy were suggestive of acute schistosomiasis or some other acute nematosis such as trichinosis. A complement-fixation test for the latter was performed to determine the possibility of a cross-reaction (Chaffee *et al.*, 1954), but the result was negative. Recently, Hsü and Hsü (1956) have demonstrated that exposure of five individuals to the Formosan strain of *S. japonicum* produced classical symptoms of acute schistosomiasis; however, eggs were never demonstrated nor was there any other evidence of maturation of the parasites. Although these findings are of interest, no parallel can be drawn with the four-year-old boy in the present study since complement-fixation studies were evidently not performed. However, it is likely that the two Puerto Ricans represent occult infections of *S. mansoni* as no sero-reactive tests for schistosomiasis were obtained with the sera of nematode-infected individuals from Trinidad, an island whose native residents have never been found infected with *S. mansoni*.

It was observed that sera from seven children infected with "myriads of adult *Trichuris trichiura*," as noted by rectal proctoscopy,[4] were all non-reactive in the complement-fixation test for schistosomiasis, which indicates that large numbers of these parasites do not influence the specificity of the test.

The serologic reactions obtained with the sera of 2 of the 8 Japanese tested may have been due to cross-reactive antibody stimulated by the infecting trematode (*P. westermani*). Eliakim and Davis (1954) have demonstrated that an antigen prepared with *Fasciola hepatica* fixed complement in low titer with the serum of schistosome-infected individuals. However, the possibility of occult schistosomiasis in these 2 patients must be considered, as in the case of the Puerto Ricans. Further studies of cases of paragonimiasis in which the possibility of schistosomiasis can be excluded are necessary for clarification.

SUMMARY

The specificity of a complement-fixation test for schistosomiasis, using as antigen a buffered saline extract of ether-extracted adult worms, was evaluated

[4] These patients were part of a group studied in a bloody diarrhea clinic (Garfinkel *et al.*, 1954).

in other helminthic infections. No serologic reactions were observed with the sera of 39 nematode-infected natives of Trinidad, an island whose natives have never been found to have schistosomiasis. The sera of only two of 69 nematode-infected Puerto Ricans without overt evidence of schistosomiasis were reactive. Two of 8 Japanese infected with *P. westermani* reacted in the test for schistosomiasis.

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RARE HISTOPATHOLOGIC ASPECTS OF MANSON'S SCHISTOSOMIASIS [1]

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Schistosomiasis is a serious and widespread endemic disease of which the pathology is still not completely determined. This paper presents some examples from autopsied cases of rare or unusual histopathologic conditions collected by this department, some of which we believe have not been previously reported.

HISTOPATHOLOGY

Esophagus. A 16-year-old male died of recurrent gastrointestinal hemorrhages. At autopsy varicose veins were found in the lower two-thirds of the esophagus and in the fundus of the stomach, one of which had ruptured causing the hemorrhages. Schistosomal cirrhosis of the liver was found, with many adult schistosomes in the lumen of the portal vein. The spleen, which weighed 1,060 gm., presented chronic splenomegaly.

Microscopic examination of the lower end of the esophagus showed a great number of lymphocytes and eosinophils in the lamina propria of the mucous membrane. The veins of the submucosa were greatly distended and one was occluded by an organizing thrombus. The lumen of another contained two isolated male worms, one female worm, and a male containing a female in the gynecophoral canal (Fig. 1). No lesions were found in the muscular layer. The adventitia showed schistosomal granulomata without eggs, and perivascular lymphocytic infiltration.

The presence of adult schistosomes in the esophagus was probably caused by a blocking of the portal blood secondary to the liver cirrhosis and the worms must have reached the varicose veins by way of the coronary vein of the stomach. Yokogawa and Morishita (Faust, 1948) have reported finding adult schistosomes in the esophageal veins of an experimentally infected horse, but we believe that the case presented here is the first recorded human instance of this condition.

Thyroid gland. This was a case of an 18-year-old female with schistosomal hepatosplenomegaly and intestinal polyposis; at autopsy granulomata were found in many organs including the liver, myocardium and lungs. The gross appearance of the thyroid gland was normal, but microscopic examination revealed schistosomal tubercles in the interfollicular fibrous tissue. In some cases the tubercles were epithelioid cells surrounded by lymphocytes; in other cases central giant cells contained egg-shells in the cytoplasm, with an encircling fibrosis beginning at the periphery of the tubercles (Fig. 2).

It has been demonstrated that the arterio-venous fistulas caused by the eggs of *S. mansoni* in the lungs may provide a route by which eggs can enter the systemic arterial system and thence disseminate to the viscera (Faria, 1956).

[1] Only *Schistosoma mansoni* is found in Brazil.

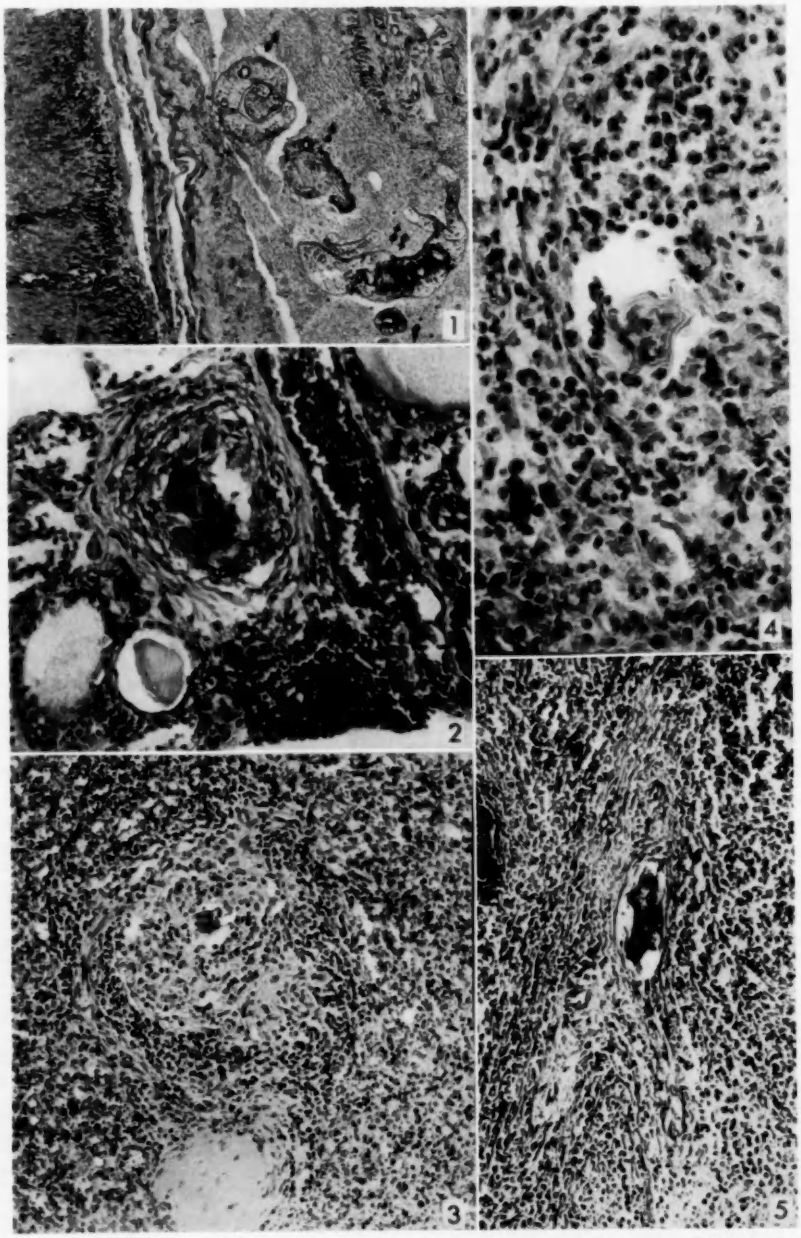

FIG. 1. Esophagus: adult schistosomes in varicose vein of the submucosa. H.E. × 65

FIG. 2. Thyroid gland: an egg-shell can be seen in a granuloma with fairly well-developed fibrous tissue encapsulation. H.E. × 180

FIG. 3. Spleen: Malpighian corpuscle with an egg surrounded by histiocytic reaction. H.E. × 200

FIG. 4. Spleen: schistosome egg in the red pulp, surrounded by eosinophils. H.E. × 360

This may account for the finding of eggs in the small arteries of the myocardium (Armbrust, 1949), the kidneys (Armbrust, 1950) and the many other foci noted in the literature. It seems probable that eggs found in the thyroid arrived by this route. Lichtenberg (1955) has recently reported a case of this type.

Spleen. In spite of the routine examination of spleen sections in countries where schistosomiasis is endemic, reports of specific lesions in that organ are rare. Sorour (1928) and Manson-Bahr (1929) failed to find schistosome eggs in spleen tissue and assumed that they were never present, although they have since been found by others, including myself. The presence of the adult worm in the spleen is still rarer, even under the favorable conditions for contamination occurring in experimental work. Brumpt and Chevallier (1931) reported that in rats only eggs could be found in the spleen, and then only in the red pulp. Koppisch (1941), however, found an adult worm in a vein in a human spleen.

Among the cases reported here eggs were found in two spleens and worms in a third. In one of the former there was a conspicuous reaction of the central area of the Malpighian bodies, including giant cell formations, and a granuloma around an egg with a miracidium was observed in one Malpighian corpuscle (Fig. 3). No other eggs were detected even after careful examination of many spleen sections. No report of a similar case was found in the available literature. It seems reasonable to suppose that in this case the egg was carried to the lymph follicle of the spleen through its central artery, as in the cases previously mentioned. The second spleen in which eggs were found showed chronic splenomegaly; eggs were in the red pulp (Fig. 4), in the lumen of the trabecular veins (Fig. 5) and in granulomata in the fibrous tissue of the trabeculae. In the third spleen three adult worms, two males and one female, were found in a large trabecular vein (Fig. 6), but no eggs were detected.

The spleen is an important organ of the portal system, and it is curious that while splenomegaly frequently occurs in schistosomiasis, lesions specific for schistosome infection are rarely found in spleen tissues. For some still undiscovered reason worms leaving the liver and the portal vein almost always migrate down the mesenteric vein, although anatomically they could as easily pass via the available splenic vein to the spleen and lay eggs in that organ. This, in fact, appears to have been the case in the instances cited.

Lungs. In schistosomal infections, especially when massive, eggs are usually carried to the lungs; adult schistosomes are much less common, although they have been found in branches of the pulmonary artery by Symmers (1904), Sorour (1928), Day (1937) and Shaw and Ghareeb (1938). This report is not concerned with such cases.

Occasionally a laboratory diagnosis of pulmonary schistosomiasis can be made by finding schistosome eggs in the sputum, but this is extremely rare. This method of diagnosis was established by Figueiredo, Leal da Costa and Mainzer according to Meira (1942) who had made similar observations. In the present study the histopathological counterpart of this condition, i.e., intrabronchial schistosomiasis, was observed in a case of schistosomal cor pulmonale. In one section an egg was seen lying on the epithelium of a large bronchus as

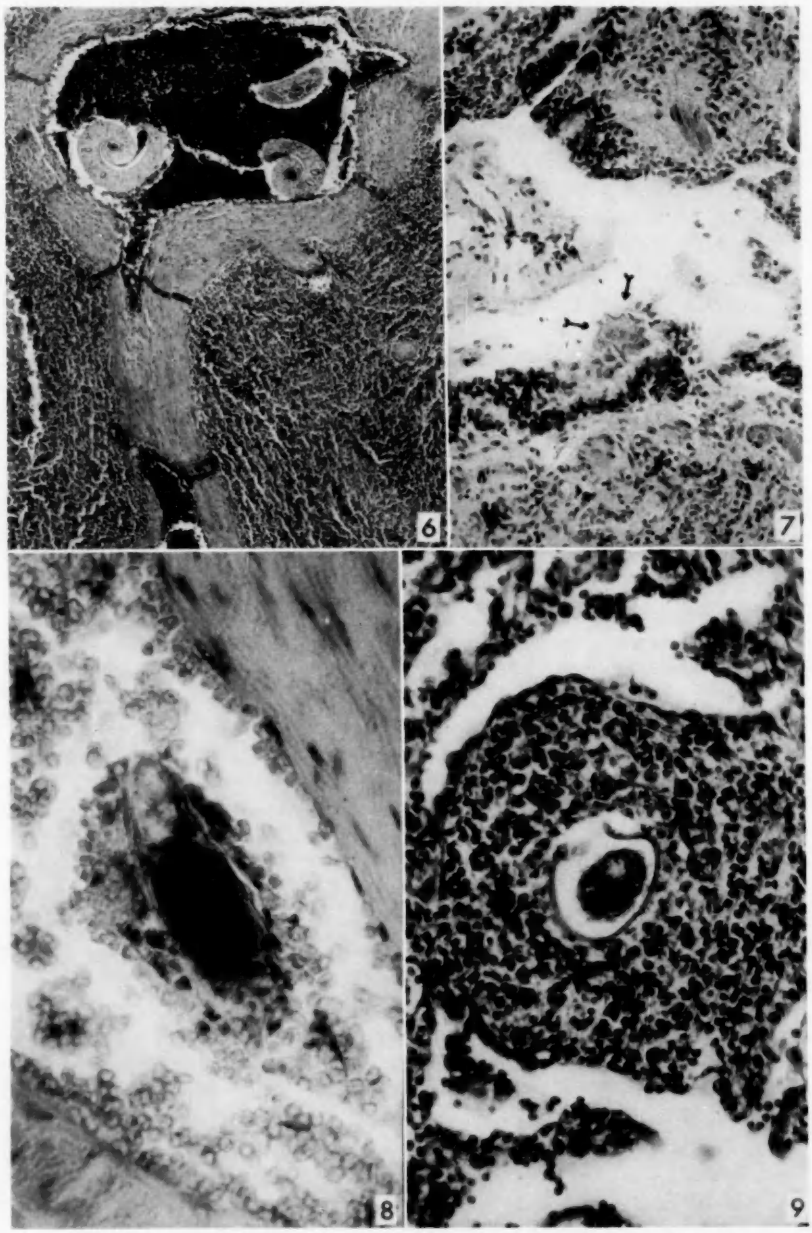

FIG. 5. Spleen: schistosome egg in the lumen of a trabecular vein. H.E. 130

FIG. 6. Spleen: schistosomes in the lumen of an intrasplenic vein. H.E. × 60

FIG. 7. Lung: an egg-shell lying on the epithelium of a bronchus (arrows). H.E. × 170

FIG. 8. A well-preserved schistosome egg in the lumen of a large branch of the pulmonary artery. The lateral spine can be seen at the lower end of the egg. H.E. × 450

FIG. 9. Lung: schistosomal granuloma containing an egg with ruptured shell. The miracidium is free, and the cilia are easily seen. H.E. × 320

though on its way to the exterior (Fig. 7); in another section an egg containing a miracidium and showing the characteristic lateral spine of *S. mansoni* was found in the lumen of a large branch of the pulmonary artery (Fig. 8). A third section disclosed a granuloma containing a ruptured egg and a liberated miracidium with cilia easily visible (Fig. 9).

Pancreas. This organ is rarely involved in schistosomiasis, although a few instances have been recorded. In the present series cases were observed in which several eggs surrounded by lymphocytes and eosinophils constituted a schistosomal pseudo-abscess (Fig. 10). In other cases an older reaction to eggs was found, with tubercles surrounded by a circular band of connective tissue at the periphery (Fig. 11). In addition, a pair of adult worms were found in an intra-pancreatic branch of the splenic vein, but in this section the body of the female was almost destroyed (Fig. 12). The presence of worms and eggs in the pancreas can probably be explained on the same basis as the similar findings in the spleen.

A major consideration related to schistosomal lesions in the pancreas would be whether resultant diabetes mellitus would be susceptible to treatment by anti-schistosome drugs. According to Rodrigues da Silva (1949), Pacha reported a patient with what seemed to be a carcinoma of the head of the pancreas which subsided after antimony therapy.

Stomach. Patients with schistosomiasis frequently complain of epigastric malaise, but few have demonstrable gastric lesions, and their discomfort may result from hepatic or neuro-vegetative disturbances. Reports of eggs in the stomach wall are extremely rare, although Rodrigues da Silva (1949) reports that Makar had seen such a case. In the present series a case was studied in which the complaints had included postprandial fullness. Microscopic examination of the mucous membrane of the stomach disclosed eggs in the lumen of the capillaries (Fig. 13) and a pair of worms in a vein of the submucosa (Fig. 14). Since hepatic cirrhosis had occurred in this case, it is probable that the presence of worms in the stomach veins may be explained on the same basis as similar findings in the esophageal veins. The eggs were undoubtedly deposited locally and reached the lumen in the same manner as in the intestine. The remainder of the stomach was normal and it does not seem likely that the patient's complaints had been caused by the presence of the eggs and worms.

Duodenum. A 17-year-old male died of hemorrhages following the rupture of varicose esophageal veins. At autopsy eggs were found in the mucous membrane of the duodenum, with a distribution and appearance similar to that in the stomach wall described above (Fig. 15). No worms were found in the vicinity but the presence of eggs can probably be explained as in the case of the invaded gastric wall.

Prostate gland. A 30-year-old male died of cachexia due to gastric carcinoma, and a schistosomal infection was found incidentally during the routine autopsy examination. Although the infection was low-grade, the prostate gland showed a few schistosomal granulomata (Fig. 16). As previously emphasized (Armbrust, 1950), while Manson's schistosomiasis is usually limited to viscera of the portal

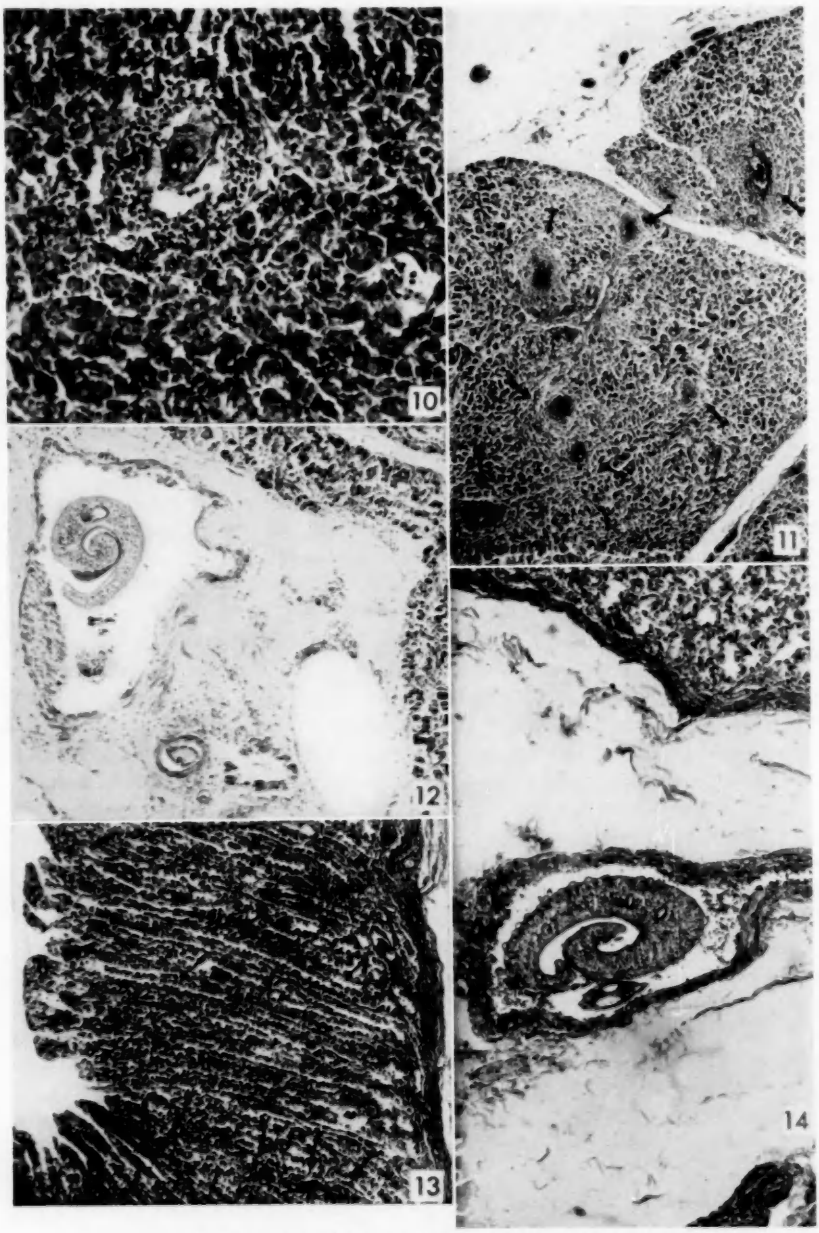

FIG. 10. Pancreas: schistosomal pseudo-abscess. H.E. × 180

FIG. 11. Pancreas: schistosmal granulomata (arrows). H.E. × 30

FIG. 12. Pancreas: schistosomes in the lumen of an intra-pancreatic branch of the splenic vein. H.E. × 70

FIG. 13. Stomach: schistosome eggs in the mucous membrane (arrows). H.E. × 70

FIG. 14. Stomach: A pair of schistosomes in the lumen of a vein in the submucosa. H.E. × 70

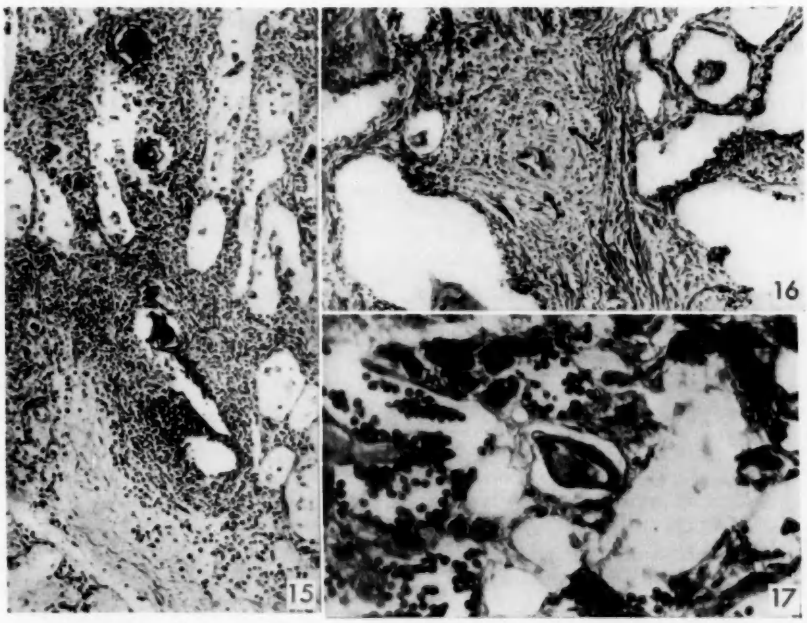

FIG. 15. Duodenum: schistosome eggs in the mucous membrane. H.E. × 130
FIG. 16. Prostate gland: schistosomal granuloma with two egg-shells. H.E. × 120
FIG. 17. Adrenal: schistosome egg in the cortex, with no perifocal inflammatory reaction. H.E. × 400

system, it is not always confined to these organs and may cause marked lesions in the genito-urinary organs.

Adrenals. Koppisch (1941) mentioned finding schistosome eggs in the adrenals but we know of no other similar reference. In one case of the present series eggs were observed in the zona fasciculata near the zona glomerulosa with no inflammation in the neighboring tissues (Fig. 17). It is interesting that some tissues (e.g., the testes and seminal vesicles) appear to tolerate schistosome eggs without reacting to their presence (Armbrust, 1950), while other tissues react very strongly. The cause of this variability in tissue reaction, which is peculiar to schistosome infections, is still to be found. An understanding of this subject is complicated by the disagreement among experienced pathologists as to whether tissue reaction is caused by the presence of dead or living schistosomal embryos.

SUMMARY

This paper describes rare and previously unreported histopathologic conditions found at autopsy in cases of Manson's schistosomiasis. Schistosome eggs were seen in the thyroid gland, spleen, lungs, pancreas, stomach, duodenum, prostate

gland and adrenals. Of particular interest were an egg found in a Malpighian corpuscle in the spleen, and an egg with ruptured shell and liberated miracidium found in a pulmonary granuloma. An egg found in the lumen of a large bronchus appeared to be the pathological counterpart of schistosome eggs in the sputum. The finding of worms in the varicose veins of the esophagus and in veins within the spleen and pancreas was a particularly rare occurrence. Theories to explain the findings of schistosome eggs and worms in the various rare locations are presented in the body of the paper.

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BANCROFTIAN FILARIASIS IN THE ETIOLOGY OF FUNICULOEPIDIDYMITIS, PERIORCHITIS AND HYDROCELE IN PUERTO RICO (STATISTICAL STUDY OF SURGICAL AND AUTOPSY MATERIAL OVER A 13-YEAR PERIOD)

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A high incidence of funiculoepididymitis, periorchitis, and hydrocele has been observed in Puerto Rico (Hoffman *et al.*, 1932; Hernández Morales and González Barrientos, 1946; Sanjurjo, 1954). While some estimates indicate that 80 per cent or more of the hydrocele cases here are filarial (Sanjurjo, 1954), there is also a growing conviction that filariasis is decreasing in frequency. Elephantiasis has become rare on the island (Koppisch, 1956, personal communication), but this condition is an unreliable index of filarial infection, since accessory factors, such as bacterial infection, may play a role in its pathogenesis (Grace, A. W. *et al.*, 1932; Pomales Lebrón, 1940). Much work has been done on microfilaremia in different population groups in Puerto Rico (Hernández Morales and González Barrientos, 1946; Bercovitz and Shwachman, 1946) but cannot be correlated with the incidence of filarial disease until more about their relationship is known (Jordan, 1955).

Most reports on genital filariasis include small numbers of cases and few contain pathological descriptions. Important contributions have been made by Ferrer (1934) who gave a clinical description of endemic genital filariasis, by O'Connor and Hulse (1935) who established the principal pathological features of filarial disease by serial sectioning of complete specimens, by Knott (1939) who described the variety of filarial lesions encountered in the genitals and by Wartmann (1947) who correlated clinical and pathological findings in U. S. soldiers in the Pacific war theater. Genital filariasis in Puerto Rico was discussed in illuminating detail by Sanjurjo (1954).

We have investigated the relationship between Bancroftian filariasis and lesions of the male intrascrotal organs in a large number of routine autopsy and surgical specimens. The first results, presented here, deal with etiological considerations and clinicopathological correlation. Studies on pathological anatomy and on pathogenesis will be presented later.

MATERIAL

During the 13-year period from January, 1940 to December 1952, 1,531 autopsies and 43,087 surgical specimens were examined at the Department. Of these, there were

	Autopsies	Surgicals
Males over 10 years of age	590	13,778
With data of intrascrotal organs	499	463
Adequate for review	436	356

The patients came from 38 institutions in Puerto Rico and in the Virgin Islands, the great majority from metropolitan San Juan. With few exceptions, they were charity patients. The autopsy cases showed high incidences of malnutrition, liver disease, infections and parasitic diseases, indicating low income and precarious general hygiene. The racial distribution resembled that described by Koppisch (1939). Age ranged from 10 to over 90 years, with a mean of 44 years in both autopsy and surgical groups. The clinical and pathological data of these cases were tabulated from the record and a review of the histological slides were undertaken.

Incidence of Funiculoepididymitis, Hydrocele and Periorchitis

Cases were assembled into two groups:

A. Funiculitis and/or epididymitis (without hydrocele or periorchitis and excluding tuberculosis and tumors).....11 autopsy, 43 surgical cases,
B. Hydrocele and periorchitis (regardless of associated pathology, excluding tuberculosis and tumors).....138 autopsy, 189 surgical cases.

Since in many cases only the funiculus or only the epididymis were available for reexamination, lesions of these organs had to be treated as a group, and only the predominant lesion was diagnosed while the number of combined lesions (Fig. 1) remained unknown. In the hydrocele group we included all cases in which a fluid collection over 10 cc. was present and those in which alterations

FIGS. 1 and 2 illustrate severe chronic endemic filarial lesions of the intrascrotal organs.

FIG. 1. Chronic funiculoepididymitis: the tunica vaginalis shows adhesions around the epididymis. The epididymis is enlarged, congested, and shows numerous dilated, coiled veins extending upward into the cord. Histologically there was chronic epididymitis and thrombophlebitis with numerous recanalized thrombi. A calcified filarial worm was identified.

FIG. 2. Chronic periorchitis and pyocele: the testis is small and atrophic. It shows adhesion and thickening of the vaginalis. The scrotal cavity is filled with a rose-colored, paste-like material consisting of inspissated pus, coagulated blood and cholesterol. This patient developed a scrotal abscess and fistula after unsuccessful hydrocele repair. Calcified worms were identified in the epididymis.

of the tunica vaginalis were observed, even when the size of the collection was not stated.

Hydrocele, lymphocele, hematocele, pyocele, and dry adhesive periorchitis were considered as related lesions, since in several cases hematocele or dry adhesive periorchitis were found contralateral to hydrocele. Pyocele was observed to develop in collections previously serous after fistula formation or operation (Fig. 2). Cases labeled lymphocele or chylocele were few and were included in the hydrocele group. In order to avoid confusion with simple testicular atrophy and fibrosis, only cases with marked tunica thickening, adhesion or histological inflammation were classified as adhesive periorchitis. The following statistics show the incidences of each group and their general composition.

A. Incidence of funiculoepididymitis:

In consecutive autopsies of males over 10 years old 2.8%
In surgical specimens of intrascrotal organs, same age 12.2%

Composition of group:	Autopsy	Surgical
Funiculitis	54.6%	74.4%
Epididymitis	45.4%	25.6%

B. Incidence of hydrocele and periorchitis:

In consecutive autopsies of males over 10 years old 28.1%
In surgical specimen of intrascrotal organs, same age 53.1%

Composition of group:	Autopsy	Surgical
Hydrocele, Lymphocele	81.2%	39.1%
Hematocele	4.3%	37.6%
Pyocele	—	12.2%
Dry adhesive periorchitis	14.5%	11.1%

The year-by-year incidence of the combined groups of funiculoepididymitis, hydrocele and periorchitis varied markedly, but did not show a definite trend within the 13-year period investigated.

Incidence of Filariasis of the Intrascrotal Organs

Diagnosis was based on the finding of one or several adult *Wuchereria*, either intact, damaged, or calcified in the histological sections of the intrascrotal organs, after systematic review.

The following groups were established:

A. Filarial funiculitis and/or epididymitis ..... 3 autopsy, 15 surgical cases
B. Filarial hydrocele or periorchitis ..... 12 autopsy, 36 surgical cases
C. Solitary filarial lesion of testis ..... 2 autopsy cases

The cases of solitary testicular lesion had a single worm, damaged or calcified, in the testicular parenchyma, in one case with thickening of the overlying tunica vaginalis.

Review of previous diagnoses showed that damaged and, especially, calcified worms in the sections had been frequently missed, so that over 30 per cent of the filarial cases were added by the review. The exact increment cannot be calculated because previous diagnoses were in part suggested and in part definite. The total of 68 cases of genital filariasis is the largest series published to date.

Since the study was made in routine material with many deficiencies, the percentages of filarial incidence constitute only the proven minimum. The probable error is commented upon in the discussion.

A. Incidence of filarial worms in the intrascrotal organs:

In consecutive autopsies of males over 10 years old 3.9%
In surgical specimen of intrascrotal organs, same age 14.3%

Associated lesions:	Autopsy	Surgical
Funiculoepididymitis	17.7%	29.4%
Hydrocele or periorchitis	70.6%	70.6%
Solitary lesion of testis	11.7%	—

B. Incidence of filarial worms in funiculoepididymitis and in hydrocele or periorchitis:

	Autopsy	Surgical
Funiculoepididymitis	27.3%	34.9%
Hydrocele or periorchitis	8.7%	18.9%

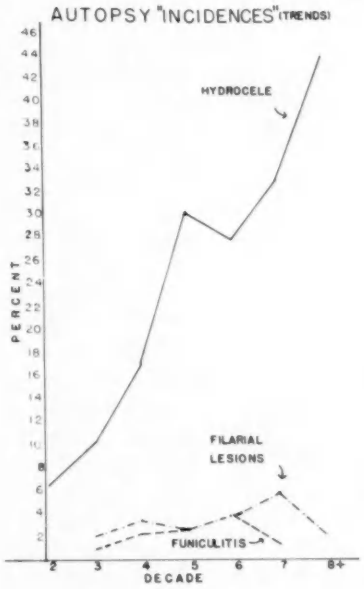
AGE DISTRIBUTION

The following tables show the age incidence of 1) funiculoepididymitis; 2) hydrocele and periorchitis; 3) filariasis of the intrascrotal organs expressed as percentage of the total number of males per decade. Because cases of unknown age were omitted in the calculation and because the age distribution for all males in the surgical material was obtained from a representative sample of 600 cases only, these incidences have to be regarded as trends rather than as accurate values.

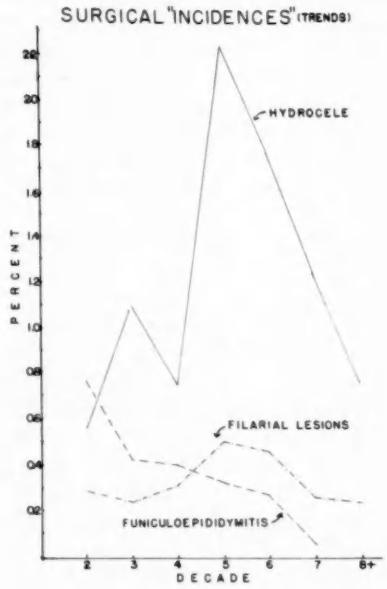
Supplementary Studies

For further clarification of the relationship between filariasis and lesions of the intrascrotal organs the following studies were performed.

A. Comparison of filaria-containing and nonfilaria-containing lesions. We compared 94 advanced cases of funiculoepididymitis or periorchitis, 39 of them filaria-containing and 55 nonfilaria-containing, with the aim of establishing secondary pathological criteria for the diagnosis of genital filariasis besides the detection of the worm. The filaria-containing cases showed a highly polymorphous picture both in degree and type of inflammation (Figs. 1 and 2). Complications or histological signs of multiple etiology were frequently present. Among these, abscess of the epididymitis, thrombophlebitis of the spermatic cord, abscess, necrosis or interstitial inflammation of the testis were the most frequent. Coincident testicular schistosomiasis was found once. Acute filarial funiculoepididymitis was usually a more homogeneous lesion and filariae were found in a



GRAPH I



GRAPH II

considerable proportion of such cases, while chronic filarial periorchitis was highly polymorphous and the localization of worms was accomplished less frequently. Many of the nonfilaria-containing lesions resembled those diagnosed as filarial. A few, however, showed histologically distinctive lesions such as mycosis, chronic granulomatous inflammation or primary orchitis. Although the cases were thoroughly studied and had multiple sections, no reliable secondary criteria for the diagnosis of filariasis could be established.

B. Etiology of hydrocele in autopsy cases. 136 cases of hydrocele or periorchitis were classified by combined clinicopathological criteria into the following groups:

1. Non-inflammatory hydrocele
   a. Due to generalized edema.
   b. Due to localized inguinoscrotal or pelvic lesions.
   c. Cause unknown.
2. Inflammatory hydrocele (periorchitis)
   a. Filarial
   b. Non-filarial
   c. Undetermined cause

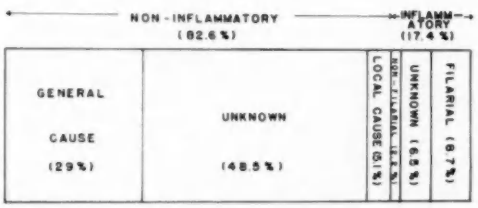
The distribution of etiologies is given in Graph 3.

C. Clinical data of filariasis in the autopsy series. Since many cases had inconspicuous lesions and others were considered as incidental to the principal disease, the accuracy of clinical diagnosis was low, and data were often fragmentary. Of 17 confirmed cases of intrascrotal filariasis only 3 were diagnosed during their hospital stay. The remaining 14 were detected at autopsy. An additional 4 cases had been diagnosed clinically as filariasis but were not confirmed at autopsy. The three confirmed and the four unconfirmed cases of "filariasis" had one or several of the classic "inflammatory" recurrent symptoms of the disease, such as lymphangitis, funiculitis, fever, lymphadenopathy, blood eosinophilia, edema or elephantiasis of the extremities or scrotum.

Four types of clinical evolution were noted among the confirmed cases of intrascrotal filariasis, namely

a. apparently asymptomatic lesion (4 cases),

ETIOLOGY OF HYDROCELE IN 138 AUTOPSIES
OF MALES OVER 10 YEARS



GRAPH III

b. gradually developing periorchitis without "inflammatory" symptoms (9 cases),

c. recurrent inflammatory episodes followed by chronic periorchitis (3 cases),

d. acute inflammatory episode terminating in death (one case of acute filarial funiculitis with associated suppurative thrombophlebitis, and multiple septic emboli. This patient died undiagnosed with lesions similar to those described by Castellani (1931) as "acute endemic funiculitis").

The division between groups a and b is somewhat artificial since a patient with a small hydrocele can be considered as practically asymptomatic. Group c was the only one showing the classical filarial syndrome.

DISCUSSION

A. Sources of error. In a revision of cases which originally were not investigated for filariasis, the sources of error obviously loom large. The incidence of intrascrotal lesions found by us may be smaller than their real proportion in the population since hydrocele is sometimes operated without tissue removal or remittance to the pathologist. The surgical statistics are based predominantly on severe and complicated genital lesions, while many of the autopsy cases have hydroceles due to terminal edema after chronic illness. Thus, neither represents the status of the population at large.

As to the incidence of filariasis, it was undoubtedly too low, since many specimens were either incomplete (i.e., hydrocele sac only) or did not have a sufficient number of histological sections. Filarial worms may have remained undetected both in apparently normal organs (solitary lesion of testis or chronic funiculitis with few worms), and in organs with obvious lesions. In the autopsy group, for instance, there were 17 intrascrotal lesions confirmed as filarial, while in 13 cases the etiology remained unknown, although they were otherwise compatible with filarial funiculoepididymitis or periorchitis. Thus, our incidences of filariae represent only the minimum detectable by routine pathological methods, but do not give a full picture of the incidence of filariasis in Puerto Rico.

B. Etiology of epididymofuniculitis, hydrocele, and periorchitis in Puerto Rico. While the autopsy incidence of hydrocele and periorchitis in the Puerto Rican male over 10 years of age (28.1%) was substantially higher than that of a similar group in Washington, D. C. (about 12%) (Peery, 1955, personal communication) and the lesions reported in our series were, on the whole, more frequent and severe than those seen in the continental U. S., the increment cannot be atributed to filarial infection alone, but must be explained by multiple etiological factors, because:

a. Tunica lesions associated with filariasis are of inflammatory type. However, a large proportion of our hydrocele cases were noninflammatory.

b. The age distribution curves reveal a lack of correlation between the incidence of filariasis and that of hydrocele, especially in the older age groups.

c. There were several cases with distinctive lesions, indicating etiological factors other than filariasis.

d. Finally, in the population group studied by us, there was a high incidence

of malnutrition, chronic liver disease, venereal infection, genital trauma, untreated hernia, or long-standing untreated prostatic hypertrophy. Susceptibility to streptococci which plays an important part in lymphangitis and elephantiasis in Puerto Rico may be a factor in funiculoepididymitis and periorchitis as well. This possibility is suggested by several cases of our series which were thoroughly searched for filariae but in which none were found, analogous to cases of endemic funiculitis with thrombophlebitis described by Paul (1950) in Ceylon.

C. Diagnosis of Filariasis. Clinical history. While early filarial disease of the U. S. soldier in the Pacific war theater usually produced a characteristic clinical syndrome ("Mumu") (King, 1944; Wartmann, 1947), cases in Puerto Rico usually seek consultation for chronic filarial lesions such as hydrocele or elephantiasis, and in many of them the classic symptoms of filariasis cannot be elicited in the history so that they simulate "Primary elephantiasis" (O'Connor and Hulse, 1935), or "Primary idiopathic hydrocele" (Sanjurjo, 1954). Our data confirm the observation suggesting that in Puerto Rico cases with a typical filarial history may be actually a minority (Danglade and Fitzgerald, 1946; McMartin, 1945). When typical symptoms are absent, diagnosis of filariasis becomes problematic, since its principal late manifestations—hydrocele, elephantiasis, or chyluria—may be produced in clinically similar forms by other etiological factors (Manson-Bahr, 1954).

Laboratory examinations. Blood smear examination, while valuable as an epidemiological index, fails in most cases of filarial disease, both early (Wartmann, 1947) and late (Jordan, 1955). The frequent negativity of blood smears has been particularly emphasized in genital filariasis (Sanjurjo, 1954; Ray, 1934). There is no agreement on the value of the skin and complement fixation tests hitherto available (Wartmann, 1947).

Pathological study. Pathological demonstration of the worm, the most accurate diagnostic method, has severe limitations. In early cases, worms were detected in 22 per cent of the specimens (Wartmann, 1947) but higher percentages have also been reported and O'Connor and Hulse (1935) do not mention failure to demonstrate the worm. On the other hand, filarial cases have been published in which the adult worm was not demonstrated (Hartz, 1950; Knott, 1939; Young, 1934) with the understanding that such a lack did not invalidate the clinical diagnosis. Inability to detect the worm may be due to "routine" examination of the specimen, or to lack of familiarity of the pathologist with partly destroyed and calcified helminthic forms. However, factors outside the control of the pathologist are more frequently the cause of failure, such as an inadequate specimen, a sparse number of worms detectable only by laborious step sectioning, or an unusual tissue reaction due to complicating factors. The number of filariae may be reduced by total worm destruction in granulomatous and necrotic foci which have been described as leaving no characteristic residual structure (Michael, 1945; Wartmann, 1947).

Several pathologists believe that filarial lesions show accessory histological changes such as endolymphangitis (Hartz, 1944) or tissue eosinophilia which permit a compatible or probable diagnosis. However, many such diagnoses

seem to be partly inspired by clinical data accompanying the specimen and by knowledge of the prevalent endemicity. In our material representing advanced lesions, we were unable to establish useful secondary criteria for diagnosing filariasis, due to the polymorphism of the lesions and to the frequent presence of associated infection, vascular disease or other complications. When typical endolymphangitis was found, worms were also detected as a rule. On the other hand late cases with calcified worms only presented great histological diversity.

Since O'Connor's method for demonstrating filariae cannot be used as a routine, we have employed a new method in recent specimens. The entire specimen is cleared by the technique utilized for detecting lymph-node metastases in carcinoma specimens, and examined with a lens or dissection microscope of the wet stage and with incident light (Figures 3 and 4).

D. Incidence of filariasis in Puerto Rico. When a disease is difficult to diagnose, it is even more difficult to rule out, hence the problems in appreciating the true morbidity of filariasis in Puerto Rico. In a survey of male prisoners, Hernández Morales and González Barrientos (1946) found microfilaremia in 4.35 per cent. After adding presumable filarial symptoms and lesions, the incidence of filariasis increased to 13.6 per cent. In a survey of inductees between 18 and 38 years of age (Bercovitz and Shwachman, 1946) microfilaremia affected an average of 3.42 per cent. Older studies yielded higher percentages (Ashford, cit. by Hernández Morales and González Barrientos, 1946). Adding 17 genital and 2 extragenital localizations of *W. bancrofti* found in our autopsy series in males over 10 years of age, we found worms in 4.4 per cent, an incidence similar to that of microfilaremia in comparable subjects. In view of the errors inherent in both the au-

FIGS. 3 and 4 illustrate visualization of filarial worms by the clearing method described in the text of the paper.

FIG. 3. Coiled filarial worm, partially calcified, showing characteristic white refringence in a cordlike structure of the spermatic cord, probably representing an occluded lymph vessel. Observe deformation and fragmentation of worm.

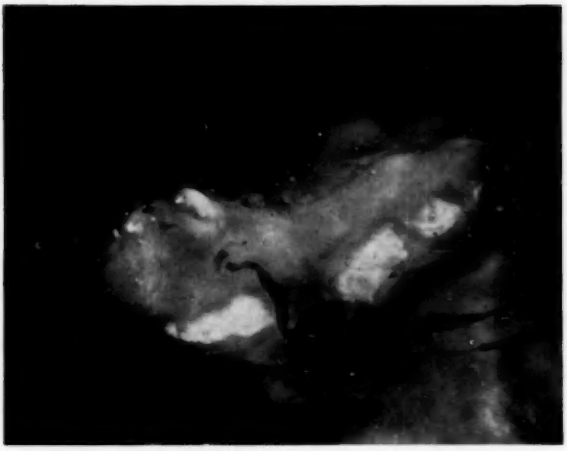


FIG. 4. Fragmented calcified filarial worms of the epididymis, showing marked refringence of cuticle and typical coiled shape. Congested venous capillaries are blackish cords. The worms are easily differentiated by virtue of their white color.

topsy and the blood-smear method, both types of incidence are probably too low. On the other hand, the incidence found by clinical approach seems to be too high, since lesions of undetermined etiology must be included in the calculations.

E. Filarial infection and filarial disease. In endemic filariasis, a fraction of the infected population presents symptoms of filarial disease. The rest show microfilaremia only. The few follow up studies available indicate that under favorable climatic conditions the asymptomatic stage of filariasis may be long lasting or permanent (Behm and Hayman, 1946; Wartman, 1947). In this study solitary or latent filarial lesions of the testis and spermatic cord were found which may represent the final stage of an asymptomatic infection. It seems probable that mild infections are frequent in Puerto Rico, as they proved to be in U. S. soldiers infected in the Pacific war theatre.

The recurrent acute inflammatory syndrome of early filariasis is today considered as allergic in nature (Rodhain, 1953; Wartman, 1947; Acton and Roo, 1930). Bacteriological studies in endemic cases have given conflicting results possibly because filariasis has been confused with streptococcal lymphangitis and cellulitis (Grace *et al.*, 1932; Pomales Lebrón, 1940). In U. S. soldiers, bacteria did not appear to play a role in early filariasis (Wartman, 1947).

On the other hand, in chronic endemic filarial lesions, complicating features, especially bacterial infections, are frequently described (Sanjurjo, 1954; McMartin, 1945; Knott, 1939; Acton and Rao, 1929). There is abundant evidence of vein lesions such as varicocele, phlebosclerosis, acute and chronic thrombophlebitis (Sanjurjo, 1954; Casile and Saccharin, 1952; Fogel and Huntington, 1944: Dejou, 1935; Sorour 1929). The lethal cases show an acute septic thrombophlebitis, and death is due to infected emboli (Castellani, 1931).

These features were well illustrated in our material, and the range of filarial lesions was unusually wide. Some of the severe surgical lesions of filarial periorchitis were so radically different from the mild autopsy cases that it was difficult to believe that both represented the same pathological entity. We have no doubt that in chronic endemic filarial lesions a great part of the alterations and symptoms are due to complicating features, especially bacterial infection or vein involvement, rather than filariasis per se. It is suggested that such complications may come about both because filarial obstruction of the lymphatics prepares the tissues for infection or vascular disease, and because other etiological factors may coincide with filarial infection (Sa de Oliveira, 1947).

Although microfilaremia in Puerto Rico decreases in frequency after the second decade (Hoffman, 1932; Bercovitz and Shwachman, 1946), filarial lesions were found distributed throughout life, with predominance in the older age groups. According to Manson-Bahr's (1954) three-stage concept of filariasis, the disease begins with an early allergic stage, followed by an symptomatic carrier stage and by a final obstructive stage which gives rise to the chronic irreversible deforming lesions after microfilaremia has disappeared or declined. While this evolution has been observed in typical cases, our data show that in endemic filariasis in Puerto Rico, one or another of the above stages is often ill defined or absent. The early manifestations may not be elicited. The late stage may produce asymptomatic residual foci only. Finally, the carrier stage may be so shortened that there is a gradual transition from recurrent acute symptoms to chronic deformity without an appreciable asymptomatic interval (O'Connor and Hulse, 1935).

These observations confirm rather than modify the three-stage concept of filariasis, which seems to us the only hypothesis offering a logical and clinically helpful interpretation of the correlations between microfilaremia and filarial disease. The need for further studies of this problem is evident.

SUMMARY AND CONCLUSIONS

Filariasis is a prominent cause of funiculoepididymitis, periorchitis and hydrocele in Puerto Rico. However, the high incidence of these lesions is related to multiple etiological factors rather than to filarial infection alone. The true proportion of filarial lesions cannot be established by the available methods.

In autopsies of Puerto Rican males over 10 years of age, filariae were found in the intrascrotal organs in 3.9 per cent and in all locations of the body in 4.4 per cent, an incidence similar to that of microfilaremia in comparable groups. For the same age groups, filariae were detected in 14.3 per cent of the surgical specimens of intrascrotal organs. The true incidence of filariasis was probably somewhat higher. Filarial lesions occurred at all ages, with predominance in the older age groups.

Endemic filarial lesions of the intrascrotal organs varied greatly in type and in severity. There were asymptomatic foci simulating a carrier state and mild chronic lesions often clinically undistinguishable from essential hydrocele except for the presence of inflammatory changes of the tunica in the operative

specimens. There were also severe lesions, usually presenting mixed or complicating etiological factors such as thrombophlebitis, bacterial superinfection or testicular damage. No secondary tissue changes pathognomonic of filariasis were found. A clearing method for the diagnostic visualization of filarial worms in tissue specimens is described.

Clinical manifestations of endemic filariasis frequently occurred in the absence of diagnostic data in the history, laboratory examinations and excised tissues. In considering the relation between filarial infection and filarial disease, support was given to the three stage concept of filariasis suggested by Manson-Bahr.

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FLEA-HOST RELATIONSHIPS OF ASSOCIATED *RATTUS* AND NATIVE WILD RODENTS IN THE SAN FRANCISCO BAY AREA OF CALIFORNIA, WITH SPECIAL REFERENCE TO PLAGUE

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An important phase in plague epidemiology is the route by which the infection can travel from the primary reservoir (wild rodents) to man. Commensal rats (*Rattus* spp.) are potential hosts, particularly in the western United States where a vast natural reservoir of enzootic plague exists in wild rodents. While the human plague infections in this country during the past 25 years have been sporadic cases of the type associated with wild rodents (Link, 1951), epidemics of the disease are usually associated with rat plague epizootics. Where ecological conditions permit the intermingling of wild rodents with commensal rats, plague infection may be transferred to the rats (Meyer and Holdenried, 1949). The infection could then pass to man in close association with plague-infected rats and threaten a recurrence of explosive outbreaks of pneumonic plague.

Rat populations in the United States are seldom found living independently of man or his influence. In rural areas, man has often provided rat habitat adjacent to or overlapping the habitat of wild rodents; refuse dumps, ditch banks and levees are only a few examples. Where rats and wild rodents coexist, interchange of their respective ectoparasites is possible as indicated by reported findings of typical wild rodent fleas found on rats. Ground-squirrel fleas were occasionally found among the collections taken from rats during the early plague work in San Francisco (Doane, 1908; Fox, 1909; McCoy and Mitzmain, 1909) and later Ventura County (Meyer and Holdenried, 1949). In more extensive surveys, Prince (1943) reported 185 sticktight fleas (*Echidnophaga gallinacea*) and 51 fleas of 17 additional species, ordinarily not found on rats, among 2,569 fleas identified from 4,188 rats (*Rattus*). The collection was made in 13 states west of the 102d meridian during a 6-year period. Miles and Wilcomb (1952) reported 12 pack-rat fleas, *Orchopeas sexdentatus*, found on 1,426 *Rattus norvegicus* trapped in western Texas, and an average of 6.9 *E. gallinacea* on these hosts.

Transfers of plague between wild and commensal rodents are probably achieved through interchange of plague-infected fleas. However, the present knowledge of flea-host relationships between the two rodent types is inadequate for evaluating the hazard of plague transmission to rats and human beings. The major objective of this study was to determine quantitatively the degree of commingling of these rodents and the incidence of their respective flea species. This is a report of a year's trapping among associated wild and commensal rodents. Data are presented on the flea relationships between the two rodent types as they were found in adjoining or overlapping habitats.

Methods and study areas. Trap lines ordinarily consisted of 25 trap sites spaced

at 20-foot intervals. Two snap traps were set at each site—one a Victor rat trap and the other a smaller, more sensitive museum special trap. All traps were baited with a paste made from rolled oats and water. The traps were usually set for 3 consecutive nights and visited the following mornings. Captured mammals were placed in flea-proof bags on which trap sites were recorded. At the laboratory, the animals were exposed to hydrocyanic acid gas and brushed for ectoparasites. Recovered fleas were identified and inoculated into laboratory animals for isolation of the plague bacillus, *Pasteurella pestis*.

All trapping was done from March 31, 1953 to April 16, 1954. Through intensive field work, 15 areas were located near San Francisco in which commensal and wild rodents appeared to be living in close association. Of these, 9 were grassy meadows near refuse dumps and 3 were marshlands bordering the San Francisco Bay. The vegetative cover on higher ground consisted of low grasses interspersed with various herbs and shrubs, while the predominant plant in the salt marsh areas was *Salicornia* sp. Areas that revealed both wild rodents and commensal rats during the first trapping were retrapped at approximately 3-month intervals. Of the 8 localities re-trapped, 3 were trapped 4 times and 5 were trapped 3 times. Rats were taken in appreciable numbers in only 4 localities: *viz.* near 2 hog farms, a grain elevator, and a refuse dump.

In order to secure rats and wild rodents in close mutual association, the traps were set where the two rodent types appeared to coexist, as along fences, embankments or watercourses contiguous with rat harborage. However, the trap lines often extended into wildtype habitat beyond the range of rats, and vice versa. Therefore, rodents of the two types which were mutually associated were selected for separate consideration of flea-host relationships. The assumption of mutual association was based on captures of the alternate rodent types within 60 feet (3 trap sites) of each other during a 3-day trapping period.

In order to compare the host specificities and to evaluate the occurrences of flea species on various host species, a numerical value was computed for each flea-host relationship. To compensate for the disproportionate numbers of hosts examined, a common denominator for each flea species was derived from the sum of its indices on all hosts (Table 2). Each number therefore represents the theoretical percentage of a flea species' occurrence on a host species, and was computed as follows:

$$\frac{\text{Specific flea index per host}}{\text{Sum of all indices for the flea species}} \times 100$$

Rodent associations and flea-host relationships. The California vole or meadow mouse, *Microtus californicus* ssp., was found in the wild type habitats of all areas trapped, and was commonly associated with commensal rats. Of 224 rats examined, 3 *Rattus rattus* ssp. and 153 *R. norvegicus* ssp. were taken in or near vegetative cover contiguous with typical rat harborages (*i.e.*, dumps, buildings, etc.).

Of these, 120 *R. norvegicus* were trapped within 60 feet of 184 captured *M. californicus* in 4 localities having heavy infestations of rats adjacent to extensive wild type habitat. An additional 33 *R. norvegicus* were taken singly or in small num-

TABLE 1

Host relationships of the fleas Malaraeus telchinum *and* Nosopsyllus fasciatus *collected from* Microtus *and* Rattus *dead-trapped within 60 feet of each other, San Francisco Bay Area, California, April 1953–Jan., 1954*

Area	Month	*Microtus californicus*					*Rattus norvegicus*				
			M. telchinum		*N. fasciatus*			*M. telchinum*		*N. fasciatus*	
		Examined	Per cent infested	Fleas per host examd	Per cent infested	Fleas per host examd	Examined	Per cent infested	Fleas per host examd	Per cent infested	Fleas per host examd
I	April	13	15.5	0.31	0	0	11	9.1	0.09	63.6	2.1
	July	13	85.0	2.4	30.7	0.54	5	60.0	1.4	60.0	5.0
II	April	5	0	0	0	0	14	0	0	35.7	0.57
	Sept.	28	28.5	0.5	14.3	0.18	16	0	0	25.0	0.50
	Dec.	21	28.5	0.86	14.3	0.14	21	4.8	0.19	19.0	1.24
III	Jan.	33	54.5	3.5	30.3	0.3	5	0	0	20.0	0.2
	Aug.	10	30.0	0.5	0	0	14	14.3	0.21	93.0	2.0
	Nov.	20	40.0	1.0	20.0	0.25	13	0	0	46.0	0.92
IV	April	2	0	0	0	0	10	0	0	40.0	1.7
	Sept.	29	83.0	6.3	31.0	0.62	11	0	0	63.6	1.27
Totals.....		184	43.4	2.1	18.5	0.26	120	5.8	0.13	45.0	1.35

bers in similar association with 59 captured *M. californicus* in 6 localities having small, localized rat infestations.

In areas where rats comprised at least 25 per cent of the total rodent capture and comparatively large numbers of both rats and meadow mice were taken together (Table 1), the rat flea, *Nosopsyllus fasciatus*, was found on 18.5 per cent of the captured *M. californicus*. Of the 210 *N. fasciatus* collected from rats and meadow mice in these areas, 48 (23 per cent) were from *M. californicus*. The occurrence of the meadow mouse flea *Malaraeus telchinum* on rats trapped concurrently was considerably less frequent in these areas; this flea was found on 5.8 per cent of the 120 *R. norvegicus* examined, and only 15 (3.8 per cent) of 407 *M. telchinum* collected were taken from rats.

In areas of trapping where rats were less than 10 per cent of the total rodents captured, the infestation rate of *M. telchinum* on rats was more than quadrupled. *M. telchinum* was found on 8 (24 per cent) of 33 *R. norvegicus* taken in these areas. Furthermore, the index of *M. telchinum* on rats was still greater where trappings resulted in captures of only one rat in wild type habitat. The 8 *R. norvegicus* harbored 41 *M. telchinum*, an average of 5.1 per rat examined. Three of these rats, taken singly, were infested with 3, 9, and 16 *M. telchinum* respectively. The prevalence of *M. telchinum* in these lightly rat-infested areas, as indicated by collections of 193 *M. telchinum* from 37 of 59 *M. californicus*, was only slightly higher than that of other areas. No rat fleas were found on the 59 associated *Microtus*, although 55 *N. fasciatus* were collected from 15 of the 33 *Rattus*.

The indices of *N. fasciatus* and *M. telchinum* on their normal hosts did not vary appreciably whether there was considerable flea exchange or little or no exchange.

(The term exchange is assumed from the data presented in this paper. Factual exchange still remains to be proved by exacting field experiments.) Furthermore, flea prevalence on atypical hosts could not be correlated with any seasonal trend. While the procedures used were not conducive to detailed analyses of seasonal flea prevalence, the records generally indicated greatest prevalence of *M. telchinum* from September to March, and minimum prevalence from April to August.

The California ground squirrel, *Citellus beecheyi* ssp., was captured in 3 trapping areas. Of 23 *C. beecheyi* examined, 15 were trapped in an unkempt park area where occasional feral rats were taken and 2 *N. fasciatus* were found in one of the *C. beecheyi*. Two ground squirrel fleas, *Diamanus montanus*, were collected, one from a harvest mouse *Reithrodontomys megalotis* ssp. and one from a meadow mouse. No rat fleas were found on 8 *C. beecheyi* trapped in close association with *R. norvegicus* near 2 city dumps. Of these, 5 were taken (2 alive) during a 3-day trapping when 12 *R. norvegicus* were trapped concurrently over the same terrain. Both species apparently used the same trails and burrows. The squirrels harbored 170 *D. montanus*, 128 *Hoplopsyllus anomalus*, and 1 *M. telchinum*; the rats harbored 9 *Xenopsylla cheopis* and 15 *N. fasciatus*. An additional 18 *N. fasciatus* were collected from 9 of 33 *M. californicus* trapped concurrently, 26 of which were hosts of 224 *M. telchinum*. These observations indicate infrequent exchange of fleas between ground squirrels and other associated rodents. In an earlier trapping of this area, however, when no *C. beecheyi* were taken, 2 *D. montanus* were collected from 2 *R. norvegicus*.

White footed mice, *Peromyscus maniculatus* ssp. were taken in 5 of the 15 areas trapped. These mice usually occurred on brushy slopes (predominantly *Rhus*, *Baccharis* and *Mimulus*) away from the usual rat harborages, and not in close association with rats. Of the 228 *P. maniculatus* examined, 178 were from 2 areas where rats occasionally were taken, however, and of these, 22 (12.4 per cent) were hosts to 28 *N. fasciatus*. These 2 areas also were sources of 2 *N. fasciatus* from a harvest mouse, *R. megalotis*, and 2 from a shrew, *Sorex* sp. Acceptance of alternate hosts was thus indicated for *N. fasciatus* in areas sparsely populated with rats. This was further shown in recoveries of 27 *N. fasciatus* from 21 *M. californicus* not closely associated with rats (*i.e.*, no rats captured within 60 feet). It is noteworthy that these include 7 *N. fasciatus* from 5 *M. californicus*, in January, in an area from which rats apparently had disappeared during the previous summer; *i.e.*, the first two trappings yielded 11 and 5 rats, respectively (Area I, Table 1), and the October and January trappings yielded no rats.

The Oriental rat flea, *X. cheopis*, was found in only 3 of the areas trapped. Of the total 37 *X. cheopis* taken, 30 were collected from 10 *R. norvegicus* and 1 *Mus musculus* ssp. closely associated with *M. californicus*, yet none was found on the *Microtus*. However, 1 *X. cheopis* was found on a *M. californicus* trapped at a time when no rats were taken.

The host relationships of fleas collected from all hosts of the 7 principal species trapped, regardless of host associations, are summarized in Table 2. While the host species were not uniformly represented in the various trappings, the collections generally indicate host preferences and host-specific tendencies of the flea

TABLE 2

Host relationships of fleas collected from small mammals snap-trapped in the San Francisco Bay Area, California, March 1953–April 1954

Mammal species	*Sorex* sp.			*Citellus beecheyi*			*Reithrodontomys megalotus*			*Peromyscus maniculatus*			*Microtus californicus*			*Rattus norvegicus*			*Mus musculus*			Totals
No. of Hosts Examined	105			23			252			225			708			221			227			1761
	Number of fleas	Fleas per host examined	Per cent occurrence†	Number of fleas	Fleas per host examined	Per cent occurrence	Number of fleas	Fleas per host examined	Per cent occurrence	Number of fleas	Fleas per host examined	Per cent occurrence	Number of fleas	Fleas per host examined	Per cent occurrence	Number of fleas	Fleas per host examined	Per cent occurrence	Number of fleas	Fleas per host examined	Per cent occurrence	
Flea species																						
Hystrichopsylla sp.							2	0.01	2.8	6	0.03	9.5	174	0.25	87.7							182
Atyphloceras multidentatus							1	*	2.0	8	0.04	17.5	116	0.16	80.5							125
Corypsylla ornata													1	*	*							1
Corrodopsylla c. obtusata	7	0.07	93.3							1	*	6.7										8
Catallagia wymani	5	0.05	7.6	1	0.04	6.1	10	0.04	6.1	38	0.17	25.8	230	0.32	48.5	7	0.03	4.5	2	0.01	1.5	293
Leptopsylla segnis										3	0.01	3.9	6	0.01	2.5	56	0.25	75.0	14	0.06	18.6	79
Diamanus montanus				741	32.2	99.96	1	*	0.01				1	*	*	2	0.01	0.03				745
Nosopsyllus fasciatus	2	0.02	1.1	2	0.09	5.7	2	0.01	0.5	28	0.12	7.2	75	0.16	9.1	283	1.28	75.0	9	0.04	2.3	401
Dactylopsylla i. franciscana																2	0.01	*				2
Opisodasys k. nesiotus							24	0.1	3.9	462	2.3	94.4	22	0.03	1.3	1	*	0.2	1	*	0.2	510
Monopsyllus w. ophidius										14	0.06	79.0	8	0.01	14.5				1	*	6.6	23
Malaraeus telchinum	2	0.02	0.05	1	0.04	0.1	35	0.14	3.4	73	0.32	7.8	2349	3.3	80.0	56	0.25	6.7	11	0.05	1.2	2527
Ctenocephalides f. felis										1	*	*				1	*	*				2
Hoplopsyllus anomalus				134	5.8	100.0																134
Xenopsylla cheopis													1	*	0.01	35	0.16	99.97	1	*	0.02	37
Total Fleas	16	0.15		879	38.2		75	0.3		634	2.8		2983	4.2		443	2.0		39	0.17		5069

* Less than 0.005

† Percent occurrence is the specific flea index per host species divided by the sum of all the indices under a particular flea species. See page 4 for method of computation and page 8 for significance and use of the value.

species found. The numerical values shown for theoretical per cent occurrences of specific flea-host relationships, computed from actual indices of flea species, compensate for the disproportionate number of hosts examined. In other words, this number represents the proportion of fleas that would infest the host if equal numbers of each host had uniform opportunity to become infested with the species of flea. These values suggest that, in addition to *N. fasciatus* and *M. telchinum*, the wild rodent flea *Catallagia wymani* may frequently be exchanged between Norway rats and wild rodents. These 3 species were found on all the principal mammal species taken. *C. wymani* also was found on each of the 3 *R. rattus* taken (see below). The percentage occurrence of *Leptopsylla segnis* on rats was 4 times that on *Mus*, and the species also was found on *Microtus* and *Peromyscus*. Interchange among wild rodent hosts, particularly *M. californicus* and *P. maniculatus*, was indicated for 3 additional species: *Atyphloceras multidentatus*, *Monopsyllus wagneri ophidius* and *Hystrichopsylla* sp.[1] Fleas which appeared to be strongly host specific were *Opisodasys keeni nesiotus*, *D. montanus*, *H. anomalus* and *X. cheopis*.

No rat fleas were found on 16 mammals of 7 species rarely taken, and not closely associated with rats. The fleas collected from these mammals, and the counties in which they were taken, were as follows:

San Francisco County: 3 *C. wymani* from 3 *R. rattus* ssp.; 1 *Nearctopsylla* sp. from 1 *Scapanus* sp.; 16 *Dactylopsylla ignota franciscana* from 1 *Thomomys* sp.

San Mateo County: 18 *D. i. franciscana* and 1 *M. telchinum* from 1 *Thomomys* sp.; 33 *Cediopsylla inaequalis inaequalis* and 1 *Hoplopsyllus glacialis affinis* from 5 *Sylvilagus bachmani* ssp.; 1 *C. wymani* and 1 *Corrodopsylla curvata obtusata* from 1 *Neurotrichus gibbsi* ssp.; 1 *O. k. nesiotus* from 1 *Peromyscus californicus* ssp.; no fleas from 2 *Reithrodontomys raviventris* ssp.

Marin County: 11 *Orchopeas sexdentatus* ssp., 1 *M. telchinum* and 1 *Hystrichopsylla* sp. from 1 *Neotoma fuscipes* ssp.

Isolations of Pasteurella pestis. The plague bacillus was isolated from a pool of 7 fleas (2 species) from *P. maniculatus* and from a pool of 58 fleas (5 species) from *M. californicus* trapped in July, 1953, on the southwest slope of San Bruno Mountain in north San Mateo County. There were no further plague findings in fleas collected from rodents trapped in this area in October, 1953 and January, 1954. However, *P. pestis* was isolated from 2 pools of fleas from *Microtus* nests collected in the area in August, 1953 and in February, 1954. These findings led to weekly live trappings which revealed a plague epizootic in March, 1954; the infection reached the contiguous *R. norvegicus* population. Details of the plague findings will be reported separately in connection with plague ecology studies which followed.

DISCUSSION

Transfers of plague from wild rodents to commensal rats in the western United States may be more common than plague surveys indicate. Although extensive

[1] A species incorrectly referred to by authors as *H. dippiei* Roths. G. P. Holland (correspondence) states that specimens taken from *Microtus californicus* in the San Francisco Bay area probably are a new species.

surveys have been conducted for locating foci of plague in wild rodents, particularly the larger, colonial rodents, relatively few surveys have been made among *Rattus* living in contact with the smaller and more common wild rodents. While plague surveillance, in varying degrees, is practiced in the larger Pacific port cities, undetected rat plague infections could occur in suburban areas where rats and wild rodents may coexist. Such infections may occur without the appearance of conspicuous rat plague epizootics, particularly in rats living mostly outside of buildings where the efficient vector flea, *X. cheopis*, is less likely to occur.

Investigations prompted by an outbreak of human plague in Los Angeles in 1924 revealed suggestive evidence of plague transfers from wild rodents to rats in 6 widely distributed localities of Los Angeles County (Dickie, 1926). Rat plague infections of undetermined source were discovered in 2 localities of Oakland, California in 1925 (U. S. Public Health Service, 1925). Of the 30 plague infections found in rats and in fleas from rats in 20 localities in California since 1929 (Public Health Reports, 1929, 1932, 1941–43, 1945), those in rural and suburban areas were, in all probability, due to contacts with wild rodent plague. Those in urban localities of the San Francisco Bay area may have been discoveries of obscure infections maintained for indefinite periods in urban rats; however the failure of interim findings during long periods of continuous surveillance in these cities suggests that even these infections may have been introduced from wild rodent plague reservoirs of surburban areas.

The first conclusive evidence of plague transfer from wild rodents to rats was brought forth by Meyer and Holdenried (1949) in an ecological study of a plague epizootic in a rural colony of rats closely associated with *C. beecheyi*. This transfer was attributed to ground squirrel fleas, since 16 *D. montanus* and 3 *H. anomalus* were collected from 37 *Rattus rattus* ssp. involved. The authors state, however, that "at the beginning of the survey, only 3 live ground squirrels remained on the ranch"—evidence that ground squirrel fleas transferred to rats when their natural hosts were decimated. In the course of their investigations in surrounding areas, these authors found only 4 *D. montanus* and 1 *H. anomalus* on 31 *R. rattus* examined, and no rat fleas among the ectoparasites identified from 53 *C. beecheyi*, "many of them taken from dumps and ranches heavily infested with rats". Eskey and Haas (1940) found only 2 rat fleas on ground squirrels and 5 ground squirrel fleas on rats in a study of "a considerable number of ground squirrels and domestic rats from refuse dumps, the vicinity of animal pens, and locations where these rodents intermingle to a certain extent". These authors further state that "many more fleas normal to native rats and mice than fleas of ground squirrels were removed from domestic rats."

The flea-host records here presented corroborate other workers' findings of infrequent flea interchange involving ground squirrels. The observations would appear to minimize the chances for plague transfers between ground squirrels and other rodents. The frequency of flea interchange here indicated between smaller rodent species, and between them and commensal rats, suggests a potential role of primary importance for native field mice in plague epidemiology. Of

particular concern in the areas studied was the flea interchange convincingly shown between *M. californicus* and *R. norvegicus*. It appears evident that *Microtus* was the principal source for *M. telchinum* found on *Rattus* since the occurrence of *M. telchinum* on *Rattus* was generally proportional to the occurrence of associated *Microtus*. This was particularly apparent where occasional rats strayed into a predominantly wild type habitat. Apparent transfers of rat fleas, *N. fasciatus*, were evidenced by their occurrences on atypical hosts following a decline of rat populations. *C. wymani* may be important because of its wide choice of hosts, and its relatively high rate of occurrence on rats. *A. multidentatus* and *Hystrichopsylla* sp. may be implicated in plague transfers among 3 small wild rodent species on which these fleas appeared with considerable indiscrimination.

On a basis of host interchange and vector ability, *N. fasciatus*, *M. telchinum* and *Hystrichopsylla* sp. would appear to be of particular concern. *N. fasciatus* has been shown to be a fairly efficient plague vector (Eskey and Haas, 1940). Though *M. telchinum* is a poor laboratory transmitter on an individual basis, Burroughs (1944) obtained positive results using large numbers of *M. telchinum*. Unpublished experiments at this laboratory have shown *Hystrichopsylla* sp. to be an efficient plague vector. Thus the latter species, prevalent in wild rodent nests, could be of prime importance in the epizootiology of wild rodent plague. Both *N. fasciatus* and *M. telchinum* could effect passage of the infection to rats, and *N. fasciatus* could be a reciprocal source of the infection to wild rodents from plague-depleted rat populations. Where ecological conditions permit only partial decimation of rodent hosts, repeated transfer of plague infection between associated populations of rats and small wild rodents seems possible.

Multimammate mice in South Africa are considered almost entirely responsible for transfers of wild rodent plague to *Rattus* (Davis, 1953). Rodent species resistant to plague are believed to be the basic plague resorvoirs in East Africa (Heisch *et al.* 1953) and in Persia (Baltazard *et al.*, 1952). California *Microtus* and *Peromyscus* were found to be highly plague-resistant in experiments performed at the San Francisco Field Station in conjunction with plague ecology studies to be reported separately.

Until their role in plague epizootiology is better known, native North American mice should not be overlooked as possible sources for human plague. Contacts of rats with inconspicuous small mammals of various species probably are more common than is generally apparent in the absence of actual trapping. It would therefore appear that small field mice should receive primary consideration in programs of plague surveillance and control.

SUMMARY

Fleas were collected and identified for one year from dead-trapped rats and wild rodents found coexisting in several localities of the San Francisco Bay region. Interchange of flea species between host species was indicated by the following principal atypical flea-host relationships: *N. fasciatus* on 18.5 per cent of *M. californicus* and on 12.4 per cent of *P. maniculatus*; *M. telchinum* on 5.8 per

cent of *R. norvegicus*. These 2 species plus *C. wymani* occurred on all principal mammal species taken. *Hystrichopsylla* sp., *A. multidentatus* and *M. w. ophidius* occurred with little discrimination on *M. californicus* and *P. maniculatus* but were not found on rats. Species indicated as quite host specific were *O. k. nesiotus*, *X. cheopis*, *D. montanus* and *H. anomalus*. Flea interchanges involving *C. beecheyi* were infrequent. *P. pestis* was isolated from 4 flea pools collected from one of the areas.

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ON THE INCIDENCE OF POISONOUS SNAKEBITES IN FLORIDA: ANALYSIS OF 241 CASES OCCURRING DURING 1954 AND 1955

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Human beings are frequently the victims of poisonous snakebites; however there are few recent studies which indicate the incidence of snakebites in this country or in any particular state. Porges (1953) estimated there were from 2,000 to 3,000 snakebite accidents each year in the United States. Andrews and Pollard (1953) analyzed 51 poisonous snakebites which occurred in Florida during 1948 and estimated that there were probably about 100 bites yearly in the state. The purpose of this study was to determine the incidence of poisonous snakebites in Florida during the years 1954 and 1955, to study some of the epidemiologic factors related to snakebites, and to calculate the per cent mortality in this group of treated cases. This problem seemed worthwhile since 47 deaths (43 in white persons and 4 in Negroes) have resulted from poisonous snakebites in Florida during the period from 1940 through 1955.

According to Carr and Goin (1955) there are 60 species of snakes found in Florida, of which 7 species or sub-species are known to be poisonous. The poisonous snakes include: the eastern diamondback rattlesnake, *Crotalus adamanteus* (undoubtedly the most dangerous because of its size and amount of venom); the canebrake rattlesnake, *Crotalus horridus atricaudatus*; the ground or pygmy rattlesnake, *Sistrurus miliarius barbouri*; the cottonmouth moccasin, *Ancistrodon piscivorus*; the copperhead moccasin, *Ancistrodon contortrix*; and the coral snakes, *Micrurus fulvius fulvius* and *Micrurus fulvius barbouri*.

METHODS

A questionnaire and a letter explaining the purpose of the study were mailed to 2,750 practicing physicians and to the administrators of 138 hospitals in Florida. The information requested included: the name, age, sex, race and occupation of the patient; the anatomical site of the snakebite; the year and month the bite occurred; the kind of snake responsible for the bite; the activity of the patient at the time of the bite; the amount of antivenin used for treatment; and the number of days the patient was hospitalized.

This analysis of poisonous snakebites is based on the questionnaires returned by 1,299 (47 per cent) of the physicians and 116 (84 per cent) of the hospitals. By comparing the hospital reports with those of the physicians duplicate cases were eliminated. Since a poisonous snakebite is a rather dramatic and infrequent medical emergency, the author feels that most physicians who treated a patient for this condition reported it. It may be assumed that most of the reports which were not returned by physicians represent "no bites treated". This impression is partially confirmed by the fact that there were 62 duplicate reports and the practitioners reported 41 cases which were not treated in hospitals. Of course,

one must realize that some minor snakebites are treated at home and never reach the attention of a physician. By using both the individual physician's reports and hospital records, I feel that a reasonable approximation of the incidence of treated snakebite cases has been obtained.

RESULTS

There was a total of 106 poisonous snakebites reported for 1954 and 135 bites for 1955—an average of 120 cases a year in Florida, and an incidence of 3.36 per 100,000 population. According to the Florida Bureau of Vital Statistics, there were three deaths from snakebites in each of the two years, so that 2.83 per cent of the treated cases died in 1954 and 2.22 per cent died in 1955, an average fatality rate of 2.5 per cent.

Of the patients who were the victims of poisonous snakebites, 179 were males and 62 were females. This high incidence in males probably reflects their greater risk of exposure to bites due to recreational habits and occupation in agriculture and out-of-doors work. There were 199 white persons bitten as compared to 41 Negroes and one Indian. Table 1 gives the number of snakebites by age groups That 49 per cent of all poisonous snakebites occurred in young people less than 20 years of age probably reflects the natural curiosity of children and their lack of knowledge about the danger of snakes. Many of these bites could have been prevented if the children had been wearing shoes and long trousers while playing in the yard, garden, fields and woods.

The occupational groups most often involved in snakebite accidents were: school children, 68; pre-school children, 48; farmers, citrus grove workers, woodsmen, and ranchers, 45; housewives, especially those who kept gardens or living in rural areas, 23; construction workers, 12; and professional or amateur snake collectors, 10. Only 7 members of the Armed Forces were bitten, although many military bases are located in Florida.

The activity of the patient at the time of the bite was not stated in 82 instances; however, it is significant that 43 children were bitten while playing in the yard or in close proximity to their places of residence. Twenty-three persons were bitten as a result of handling a poisonous snake. Of the persons reported as

TABLE 1

Poisonous snakebites in Florida by age distribution

Age group	No. bites
0– 9 yrs.	64
10–19 yrs.	55
20–29 yrs.	32
30–39 yrs.	38
40–49 yrs.	18
50–59 yrs.	13
60–+ yrs.	21
Total	241

TABLE 2

Month poisonous snakebites occurred in Florida, 1954–1955

Month	No. bites	Month	No. bites
January	7	July	35
February	6	August	33
March	13	September	38
April	25	October	16
May	27	November	7
June	28	December	6

bitten while engaged in recreation, 17 were fishing, 2 were swimming and 2 were hunting.

Table 2 lists the number of snakebites by the month of occurrence, and reveals a striking seasonal distribution, in keeping with the habits of snakes which are relatively quiescent during the winter months. There is a gradual increase in the number of bites beginning in March and reaching a peak from July through September. Contrary to popular opinion, most snakebites do not occur during the hunting season.

As seen in Table 3, rattlesnakes accounted for 119, or about 50 per cent of the 241 snakebites reported. Cottonmouth moccasins were responsible for 50 cases of envenomation; whereas copperhead moccasins and coral snakes were only rarely involved. Copperheads are found only in the northern part of the state. Coral snakes, being more secretive and less aggressive than the pit vipers, accounted for few human bites. The two cobra bites were inflicted on a professional snake handler who extracts cobra venom for medical use. Parrish and Pollard (1957) in a clinical study of 60 poisionous snakebites found that the large rattlesnake species and cottonmouth moccasins more frequently produced cases of severe venenation than do copperhead moccasins and pygmy rattlers. In agreement with this finding is the fact that five of the six deaths reported in this series resulted from rattlesnake bites.

Poisonous snakebites occurred more frequently on the extremities than else-

TABLE 3

Kind of poisonous snakes inflicting bites in Florida, 1954–1955

Kind of snake	Number of bites	Per cent
Ground rattlesnake	50	21
Eastern diamond-back rattlesnake	11	5
Rattlesnake, species undetermined	58	24
Cottonmouth moccasin	50	21
Copperhead moccasin	6	2
Coral snake	5	2
Pit viper, species unknown	5	2
Cobra	2	1
Unknown	54	22
Total	241	100

where, for 43.5 per cent of these bites were on the upper extremities and 47 per cent were on the lower extremities. Therefore, more than 90 per cent of the bites occurred on the extremities. The anatomical site of the bite was not stated in 7.9 per cent of the cases. Swartzwelder (1950) in a study of 306 cases of snakebite admitted to the Charity Hospital in New Orleans also found a high percentage of bites on the extremities. One of the patients was bitten on the forehead while peering into a box which contained a "pet" rattlesnake. Another patient was bitten on the buttock while squatting to light a camp fire. The bites which occurred on the toes and feet (22 per cent) might have been prevented if the patient had been wearing shoes. It is fortunate that most snake bites are inflicted on the extremities, because this site allows for the use of a tourniquet and local surgical therapy. In general, bites on the body and face are more serious than those on the extremities, because the venom is more rapidly absorbed into the systemic circulation from these sites.

Much can be learned from the course of hospitalization of patients treated for poisonous snakebites. Wood, Hoback and Green (1955) have devised an excellent clinical classification of the severity of pit viper poisoning based on the signs and symptoms of venenation and the subsequent hospital course of the patient. They classify bites as minimal, moderate and severe venenation. Forty-one of the patients in this series required no hospitalization, and 58 required only one day of observation and treatment. The majority of these patients experienced either no venenation or minimal venenation. It is possible for a snake to bite a person without injecting venom into the body. Ninety-five (39 per cent) of the patients required from 2 to 4 days of hospitalization. In most instances these patients probably suffered moderate venenation. On the other hand, forty-seven (20 per cent) of the patients were hospitalized from 5 to 30 days. They undoubtedly represent those with severe venenation and/or complications. Three of these patients had an extremity amputated, and five others required a digit removed because of infection or gangrene. The morbidity resulting from snakebite wounds is not generally appreciated.

The amount of Polyvalent Antivenin Crotalidae (Wyeth) used in treating this series of snakebites deserves some comment. The administration of antivenin was as follows: none was given to 44 patients; one ampoule was given to 95 patients; two ampoules were given to 46 patients; 3 ampoules were given to 15 patients; and four or more ampoules were given to only 9 patients. The manufacturers recommend that at least five ampoules be given to all patients with a serious snakebite. Apparently most of the patients with minimal venenation were adequately treated with one or two ampoules of Antivenin; however, only nine of approximately forty-seven patients with severe venenation received the recommended amount of antivenin. Most of the patients with prolonged hospitalization received either no antivenin or only one or two ampoules.

SUMMARY

A questionnaire was sent to the practicing physicians and hospitals in Florida requesting them to report all the poisonous snakebites they treated during the

calendar years 1954 and 1955. They reported 106 bites treated during 1954 and 135 during 1955—an average of 120 bites per year. There were three deaths from snake venom poisoning during 1954 and three during 1955. There was an average fatality rate of 2.5 per cent for this group of treated snakebites.

Snakebites occurred more frequently during the spring and summer months. Rattlesnakes were responsible for about 50 per cent of the 241 bites. More than 90 per cent of the bites were inflicted on the extremities. Forty-nine per cent of the bites occurred in young people less than 20 years of age.

It was estimated that 41 per cent of these patients had minimal venenation, 39 per cent had moderate venenation, and 20 per cent had severe venenation. Apparently the patients with severe venenation did not receive the recommended amount of antivenin.

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BOOK REVIEWS

Virus. Die Geschichte vom geborgten Leben, by WOLFHARD WEIDEL, Direktor am Max-Planck-Institut für Biologie in Tübingen. 186 pp. (Verständliche Wissenschaft, Band 60). Berlin, Springer-Verlag. DM 7.80.

This book is volume 60 of a series written to inform the educated general reader, not the specialist. The series is called "Verständliche Wissenschaft" (clear, comprehensible science), but the style defeats this aim. The sentences are overly long, and their structure is excessively complicated. Some of the elaborate metaphors ("Fliessbänder laufen auf vollen Touren") assume the role of official technical terms; they will be understood easily by the virologist, but they do not serve to make the information easily accessible to others who might be interested. It is difficult to see what audience this book will find in the United States. The qualities of the text require that the reader have an advanced knowledge of German and considerable curiosity about viruses.

The content is of good quality: the up-to-date information is systematically presented, and the position of the virus in the biological system is elucidated qualitatively. The general characteristics of the viruses are described plastically through a description of fundamental experiments. The book is superbly printed and illustrated.

One could justly complain about the terminology and explanation of certain catch phrases ("Metaphysiker unter den Urzeugern" and "Welt ansschauliches Gemälde") and about disappointing omissions of material one would expect to find. In the chapter "Mechanismus oder Wesen", the term "Wesen" is not discussed and the explanation of "Mechanismus" is rather bizarre. Because of the omission of the names of investigators and of the dates of

certain steps in the progress of virology, some of the perspective and the romance of this development is lost.

For the time being, the general reader in America will probably gain more from the reading of F. M. Burnet's *Viruses and Man*, which deals with this subject clearly and comprehensibly.

K. F. MEYER

The Future of Arid Lands, Papers and Recommendations from the International Arid Lands Meetings, edited by GILBERT F. WHITE. Publication Number 43 of the American Association for the Advancement of Science. 464 pp. illustrated. Wash., D. C.: American Association for the Advancement of Science, 1956. Regular, $6.75; prepaid to AAAS Members, $5.75.

The significance of this volume can best be understood from the character of the meeting which it summarizes and commemorates. In April, 1955, there was held in Albuquerque, New Mexico, a world wide conference on arid land use, organized under the able leadership of the AAAS, with the University of New Mexico as host. To it were brought some of the ablest experts in arid-lands history, use techniques and research, from all over the world. It was a notably successful attempt to pool world-wide experience and information on the age-old efforts of men to occupy and live on arid and semi-arid territory, which makes up nearly a third of the land surface of the earth. A host of disciplines were represented by experts drawn from seventeen countries. For a week they lived together, ate and drank and relaxed together, took field trips and attended meetings. Between the public sessions, the delegates and interested visitors broke up into organized discussion groups and symposia for intensive exchange of experience and information on specific problems. The underlying purpose of this conference was to define more clearly the fields and the direction of basic research needed to avoid the disasters which have in the past overwhelmed civilizations based on arid and semi-arid areas.

For those who attended the conference, the present volume, with its thirty-four essays there presented, will be a valuable summary of the topics discussed and a guide to further research. For anyone desiring a better understanding of the problems which confront populations now living on arid and semi-arid land, where so much of the world's unrest originates, this series of essays will provide a fascinating survey. The bibliographies provided with each essay will open the door to more intensive study if that is desired. A vast amount of pertinent source material is scattered through the literature of so many disciplines, that it might be completely lost to unspecialized readers without this aid. Type, paper, format and editing is excellent, and as a typical amateur in the field, the reviewer can vouch for the fascination which the volume offers. The only criticism might be the lack of an appended glossary by which the scientific names of plants could be converted into more familiar terms for the layman.

HAROLD BRADLEY

African Ixodoidea. I. Ticks of the Sudan (with special reference to Equatoria Province and with preliminary review of the genera *Boophilus*, *Margaropus* and *Hyalomma*), by HARRY HOOGSTRAAL, Head, Department of Medical Zoology, U. S. Naval Medical Research Unit No. 3, Cairo, Egypt. Research Report NM 005 050.29-07 Department of the Navy, Bureau of Medicine and Surgery. 1st edition. 1101 pp., illustrated with 372 figures on 103 plates. Washington, D. C., U. S. Government Printing Office, 1956.

At the turn of the century the letters "R.A.M.C." were frequently appended to the names of authors of faunistic papers and books in the English language. However, apart from occasional papers in the *J. R. Army med. Cps.*, that was about as far as official connections with the British armed forces got; authors had to find their own publishers! More recently those concerned with actual or potential disease vectors throughout the world have become more and more accustomed to finding the imprimatur of the U. S. Navy on a varied

and extensive series of English language publications on purely terrestrial faunas of one kind or another. Perusal of these works usually reveals that the author or authors have had the most extraordinary opportunities and enviable facilities afforded them for the specific research reported on, by the research agencies of the U. S. Navy, for the widest variety of research projects. Hoogstrall's book is a worthy example of this—and one can think of few arthropod faunistic studies less concerned with the marine element than Ticks of the Sudan! Biologists everywhere owe an increasing debt to the breadth of outlook of the U. S. Navy in its research program.

It is a pity, however, that for publication recourse has been had, in the case of this book, to modern litho-reproduction of typescript with no reduction of the size of the letters. The book is bulky (10¼ x 8 x 2⅛ inches) and heavy (4 lbs. 13 ozs.) and totals a large number of pages. The use of orthodox printing with carefully selected type faces would have produced a book of more manageable size; the bibliography occupies 190 pages with references at the rate of about 9.9 titles per page! One notes, however, the excellence of the reproduction of the figures, on misnamed "plates", which are reproduced by the same process as the text and are in fact an integral part of the text and not separately printed inserts.

"The Sudan" of this book is the area previously known as the Anglo-Egyptian Sudan; it does not include French territories with the same name. The Sudan, in this sense, comprises areas representative of a great many of the Ethiopian sub-regions and it is not surprising that most of the important species of African ticks are found within its boundaries.

A feature of the literature on ticks is that it is scattered in a large number of journals and other works. A major achievement of this book is the masterly way in which the information in this vast and polyglot literature has been assembled and economically set down. The review of the literature has been as wide as the geographical distribution of the species dealt with in the book; the book is, in fact as nearly complete a reference book to the species mentioned therein as could be assembled during the progress of a project in a geographically limited area. In reviewing the literature the author has been properly critical and some oft repeated misstatements are detected and corrected.

Aspects covered include identification, distribution, anatomy, morphology, biology, ecology, host relationships as well as significance as disease vectors, and control of ticks. The amount of information offered ranges from over 60 pages on *Ornithodoros moubata* to a tenth of that amount in the case of species of lesser importance. Considerable attention is given to "identification" but there are no detailed statements of synonomy. Perhaps this is an advantage from the point of view of field workers and lest this comment should be misinterpreted it should be mentioned that the book is based, first on extensive field collections made by the author and second on his personal examination of much other material of earlier workers now lodged in various museums. This material included the types of Nuttall and Warburton which are now lodged in the British Museum (Natural History), London, and no longer in the Molteno Institute of Parasitology at Cambridge University.

The fact that a new species, *Margaropus reidi* on pp. 577–582 with figs. on pl. LXVIII, p. 576, host:giraffe, is described in the book makes it unfortunate that the title-page gives the "date of publication" as "1956"; sound taxonomic practice should call for a greater precision in dating. It is a pity that, in a work with so much extra-Sudanese data in it, a foot note reading:— "Characters in this key [to genera] do not necessarily apply to species within the same genera from areas outside the Sudan." should appear (p. 39) even if the reader is likely to realize that this is really a form of taxonomic risks insurance rather than a statement of non-utility! This needs to be emphasized because one feels that this volume will prove a milestone in Ixodoidology and as such will be a great boon to tick-workers generally and students of the African fauna in particular; other volumes are promised to follow.

The inclusion of a section on the "climate and biotic features of the Sudan" is useful even if its contents have been culled from other published sources; zoologists often seek such information and often find it a little difficult to locate.

The list of acknowledgements shows that the author has sought advice and help widely

and that both have been freely given to him. This is yet another assurance of the reliability of the book.

JOHN SMART

The Happy Life of a Doctor, by ROGER I. LEE, M.D. 1st edition, 279 pages illustrated. Boston, Little, Brown & Company, 1956. $4.00

If there is a Boston attitude toward medicine which distinguishes it from that of New York or Baltimore, Dr. Lee is an excellent representative of its three reputed characteristics —individualism, iconoclasm and idealism. As an idealistic and somewhat heretical individualist, he laments the necessity for so much organization in the profession; for group practice by associated yet isolated specialists, with their collective results; and for the mechanization of diagnosis to the point of despotic control by the laboratory. Still, he realizes that the day of the "great clinician" is over; no one now attempts to cover the whole field of internal medicine. He is not very clear how he would reconcile these incompatibles, but he suggests the cult of the "specialism of competence."

He hates war and did not like the army medicine of which he had a good dose, since it was predominantly administrative, provided too little opportunity for research, and worst of all, its fixed salaries and hierarchies tended to deprive doctors of an essential stimulus to perfection. On the other hand, how doctors are to be paid is a dilemma. "The greatest deterrent to the complete joy of medical practice is the medical fee." He doesn't approve of compulsory insurance since it intrudes between the doctor and his patient; nor of the Mayo Clinic's method of investigating the patient's financial situation and charging accordingly; nor of his own highly personal system which in the opinion of his colleagues, makes him a "menace to the profession." Some sort of insurance plan must be worked out conserving the direct relationship between doctor and patient, the latter's free choice of a physician, and the concept of the patient as a living person, whose complexity is the joy of medicine.

Harvard is his sun, about which he has revolved since his student days as one of its more rotund and important satellites. It is a solar system which merits study, including the not infrequent comets in their erratic orbits. (He takes a little time off to explain how Harvard is run.) It is an interesting, thought-provoking and amusing book, full of anecdotes and sayings, with pencil sketches of the men of his time. He is proud of his profession. There are many systems of law and many religions, but human physiology and human disease are the same the world over. It is the universality of medicine which makes it unlike all the other professions, and Dr. Lee succeeds in transmitting to the reader a sense of the happiness he experienced in being a doctor.

L. W. HACKETT

Venoms. Papers presented at the First International Conference on Venoms December 27–30, 1954, at the Annual Meeting of the American Association for the Advancement of Science, Berkeley, California. Edited by Eleanor E. Buckley, Wyeth Laboratories, Philadelphia, and Nandor Porges, U. S. Department of Agriculture, Philadelphia. Publication No. 44 of the American Association for the Advancement of Science. 1st edition, 480 pages, illustrated. Washington, D. C., The American Association for the Advancement of Science, 1956. $9.50; $8.25 to AAAS members.

The International Conference on Venoms was very stimulating to those of us who attended. Not that the material presented was new, in fact much of it had appeared previously in the literature; nor because all those who presented papers were venomologists, but because of the exchange of ideas that took place. This exchange of thoughts is the heart of any symposium and would be of real value to those who did not have the privilege of attending. In this respect "Venoms" will fail to satisfy its readers. It is regrettable that the discussions which followed the presentation of the papers were not included as is frequently done

in publishing the proceedings of a meeting of this kind. A good example of a symposium adequately reported is given in "Cold Injury, Transactions of the Second Conference, etc., 1952", sponsored by the Josiah Macy, Jr. Foundation. It is gratifying to see Mr. Klauber's remarks (p. 273) included. The comparative venomologist has recognized for some time the need of good taxonomy and the frightful waste of money in the failure of many workers to recognize the importance among species, and larger group differences, in venoms. On the other hand, it is regrettable that the volume should have been published without a challenge to the biased and incorrect statements made in what was supposed to be discussions of the value of cryotherapy in the control of venoms.

The many hours of effort and the expense involved in preparing "Venoms" are greatly appreciated, as is also the compilation of all this information under one cover. Should we be fortunate enough to have another conference in the near future, it might be wise to screen the participants more carefully and have more papers on comparative venomology—the area showing the greatest need at present.

H. L. STAHNKE

Climate and Economic Development in the Tropics, by DOUGLAS H. K. LEE. Published for the Council on Foreign Relations. 1st edition, 182 pages. New York, Harper & Brothers, 1957.

This report of some 180 pages is the result of the discussions of a Study Group on Climate and Economic Development in the Tropics set up in 1953 by the Council on Foreign Relations and composed of men representing a great variety of skills and interests. The object was to examine the direct and indirect effects of climate on productivity in the tropics. The book concerns itself almost exclusively with the wet tropics with their jungles, malaria and high humidities; it would have achieved a better balance had a chapter been devoted to the latent potentialities of the vast semiarid regions. The role of climate is explored in relation to crop production, animal husbandry, industry and human efficiency, and the known methods of overcoming obstacles to progress in underdeveloped lands are carefully weighed, from the applications of genetics and disease prevention to nuclear power and social measures. It is a little surprising to find the effect of climate on human physiology, under the proposed conditions of agricultural and industrial speed-up in the tropics, handled with such confidence. Ellsworth Huntington's conclusions as to a causal relationship between climate and human achievement are given short shrift, and there is no hint that there are serious gaps in our knowledge of this important factor. The rapporteur, who is Professor of Physiological Climatology at Johns Hopkins, is able to state that "no permanent deleterious effects occur in healthy persons living under natural tropical conditions." It is well brought out that, playing an almost dominant part in the total picture, there are nonclimatic factors such as land tenure, agricultural methods, economic status and social restraints. There must be integration of effort in the interrelated fields of health, education, agriculture, power and communications. Existing knowledge if properly applied would go a long way toward solving many difficult problems, but the people who receive financial and technical aid must be brought to desire it. Social evolution is as inevitable as organic evolution, but it cannot be hurried. The next step should perhaps be to organize tropical climate research institutes, located in the tropics, for continued study of the "broad problem of the influence of tropical climates upon indigenous populations and the development progress, rather than just on temperate climate sojourners in the tropics." The book represents a commendable prescription for a synthetic handling of the complex situation.

L. W. HACKETT

Current Therapy 1957, edited by HOWARD F. CONN, M.D. Consulting Editors: George E. Burch; M. Edward Davis; Vincent J. Derbes; Garfield G. Duncan; Hugh J. Jewett; Perrin H. Long; Clarence S. Livingood; H. Houston Merritt; Walter L. Palmer; Hobart A. Reimann; Cyrus C. Surgis; Robert H. Williams. 1st edition, 731 pages. Philadelphia, W. B. Saunders Company, 1957. $11.00.

This large book, ninth in the series, achieves a very complete, authoritative and lucid presentation of current thinking on the treatment and management of the whole imposing roll of human diseases from allergies to zoster; it is not concerned at all with diagnosis. It is made up of contributions from 300 well-known physicians who seem to have been very carefully drawn from every section of the U. S. (32 states, the District of Columbia and Puerto Rico) and from five foreign countries. While not compiled with the tropical practitioner in mind, the book nevertheless gives up-to-date coverage of the treatment and prophylaxis of "infectious" diseases, an elastic category which is here stretched to include botulism and mushroom poisoning as well as the animal parasites. In this section and throughout the book, the names of many authors well-known to almost every reader of medical scientific literature are an earnest of the high quality of the collected articles. Unavoidable in dealing with an art as empirical as therapy, points of controversy exist in every field, and while an attempt is made to present reputable opinion on both sides of the more resounding disagreements, as for example in diabetes, the minor conflicts are usually settled out of hand by the dictum of the particular person invited to collaborate. It could hardly be done otherwise, and by careful selection of authorities the editors seem to have successfully avoided fanaticism and opinionated obstinacy. On the whole the book is well balanced, up-to-date, conservative in the best sense, and should be of considerable reference value to the practicing physician who despairs of attempting to cover and compile notes from the periodical literature in this vast field.

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NOTICE TO CONTRIBUTORS

The Journal welcomes papers by members or nonmembers which contribute to the purpose of the American Society of Tropical Medicine and Hygiene. This is the advancement of knowledge of tropical and subtropical medicine and hygiene through investigation, prevention, treatment and control of diseases and deficiencies. It draws upon medicine, biology, public health, nursing, engineering and allied fields of research and application.

The American Journal of Tropical Medicine and Hygiene combines the American Journal of Tropical Medicine and the Journal of the National Malaria Society and therefore has a special concern with the investigation, therapy and control of malaria in all parts of the world, as well as a broad interest in general parasitology and in arthropod borne diseases.

Papers are ordinarily published in the order of reception, which will entail a certain delay in their appearance because of the contractual limitation on the number of journal pages available in any year. Accepted papers which raise no questions of scientific priority may however secure earlier publication if the author or the institution sponsoring the paper will pay the publication cost, an estimate of which the Editor will be glad to obtain from the publishers. Such a subvention will be used to purchase extra pages, in excess of the regular page allocation for any issue of the Journal.

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Papers should be written in *English.*

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Tables should be typed on separate sheets and be serially numbered.

Figures and *photographs* should be enclosed separately and protected with cardboard to avoid creasing in the mails. Only glossy prints can be used. Line drawings and graphs should be submitted on white paper or tracing cloth, and not on cross section paper. Colored illustrations can only be reproduced at the expense of the author.

References should be arranged in alphabetic order according to authorship, with the date following the author's name, e.g.:

Snyder, T., and Meleney, H., 1941. The excystation of *Endamoeba histolytica* in bacteriologically sterile media, *Am. J. Trop. Med.* **21**: 63–73.

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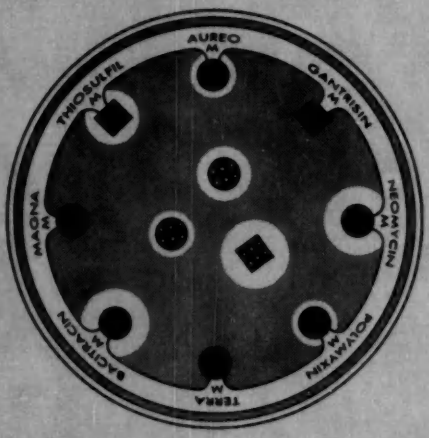

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